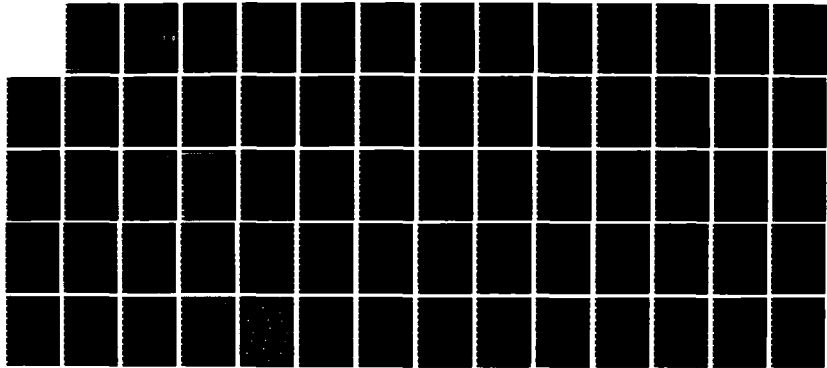CONSTRUCTION OF NEW POTENTIAL REACTIVATORS OF
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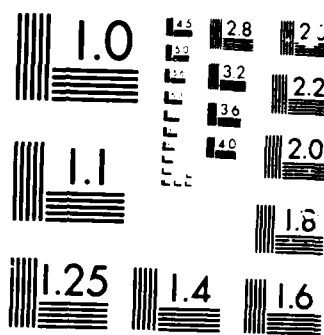UNCLASSIFIED NOV 82 DAMD77-81-C-1160 F/G 6/15 NL

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Substitution of F for H in the
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Syn-3-Fluoro-4-pyridinecarboxaldehyde oxime methiodide (3-F-4-PAM), a new potential reactivator of phosphonylated acetylcholinesterase, has been synthesized and characterized by classical organic chemical and structural methods. The pKa of 3-F-4-PAM is 8.18, which represents a lowering of the pKa value of 4-PAM (pKa 8.6) by 0.4 pKa units, due

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to the electron-withdrawing effect of the F atom. The pKa value of 3-F-4-PAM equals that of the potent reactivator TMB-4, and thus the reactivator performance of 3-F-4-PAM will constitute a test of the structural parameters of 3-F-4-PAM versus those of TMB-4 as well as those of 2-PAM. In a chemical test relevant to reactivator performance it was found that the Beckman decomposition rate of 3-F-4-PAM-benzoate is accelerated relative to that of 4-PAM-benzoate, also due to the electron-withdrawing effect of the F atom. Systematic comparative studies of the NMR and UV absorption spectra of 2-PAM, 4-PAM, and 3-F-4-PAM were carried out to help build a data base for eventual interpretation of comparative reactivator behavior. Initial chemical synthesis steps towards other F-PAM compounds were also performed.

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Note to reader: The proton NMR spectra reproduced in this report include the operator's handwritten notations of instrumental set-tings and other incidental notations at the righthand side of each spectra. Upon photographic reduction of the spectra to conventional page size, some of these notations may be difficult to read or il-legible. However, they are not essential to the content of the re-port and in all cases NMR data subjected to interpretation in this report have been entered in typography to insure legibility.

List of Figures

List of Figures (continued)

SUMMARY

The purpose of this work is to chemically synthesize and characterize new quaternary pyridinecarboxaldehyde oxime halides (PAM compounds) which by way of strategic F-substitution in their pyridine rings have the potential of being rapid reactivators of phosphonylated acetylcholinesterase. The design of these new compounds (F-PAM's) is based on current empirical knowledge of the chemical parameters which are crucial to the reactivation process (e.g., molecular stereochemistry and oxime pKa), and the ultimate performance of these F-PAM's as reactivators of acetylcholinesterase will constitute explicit tests of those parameters.

During this initial year of work we successfully synthesized syn-3-Fluoro-4-pyridinecarboxaldehyde oxime methiodide (syn-3-F-4-PAM) and have rigorously characterized this compound with respect to its pertinent physical properties. Synthesis was accomplished by a sequence of classical organic reactions starting with commercial 2-Amino-4-picoline and progressing to 3-Fluoro-4-picoline. The latter compound was then converted to the corresponding 3-F-4-pyridinecarboxaldehyde oxime by the Markovac-Stevens-Ash-Hackley method (27% yield) followed by quaternization with methyl iodide to give 3-F-4-PAM (91%). Structure proof was achieved by the usual methods of elemental analysis, mass spectroscopy, and NMR spectroscopy, and the nonquaternized parent oxime was additionally subjected to X-ray crystal structural analysis which showed, among other details, that the oxime group has the syn-geometry.

In contrast to 4-PAM, whose pKa is 8.6, the pKa of 3-F-4-PAM was found to be 8.18. Thus, substitution of F for H in the 3-position of the pyridine ring of 4-PAM lowers the pKa by 0.4 units.   The pKa of 3-F-4-PAM therewith equals that of the known potent reactivator TMB-4 (pKa 8.2), and by pKa criteria 3-F-4-PAM is qualified to be a rapid reactivator. Performance as a reactivator will therefore constitute a direct test of the efficiency of the 3-F-4-PAM structure (i.e., a mono 4-oxime configuration) versus the more complex TMB-4 structure (duplex 4-oxime).

Chemical (nonenzymatic) reactivity tests proposed in the literature by Hagedorn and coworkers for potential reactivators were carried out on 3-F-4-PAM and 4-PAM for comparison. We found that the proposed test for nucleophilic reactivity, i.e., reaction of the potential reactivator with triethyl phosphate (aqueous, pH 7.40), is too slow to be pragmatically useful. This applies to the reactivity of 2-PAM as well as that of abovementioned compounds. By contrast we found that the proposed test for Beckman decomposition reactivity is practicable. For this test we synthesized the benzoate ester of 3-F-4-PAM and additionally the corresponding ester of 4-PAM for comparison. Structure proof was achieved by elemental analysis

and NMR spectroscopy. Decomposition of these benzoates, monitored by UV absorption decay, proceeds smoothly in aqueous buffer solutions at pH 8.05. The decomposition rate of 3-F-4-PAM-benzoate is 5 times faster than that of 4-PAM-benzoate (half-lives of 12 and 60 minutes, respectively). Our prediction that Beckman elimination (decomposition) would be accelerated by the presence of the F atom in the pyridine 3-position is confirmed by this finding. Facile Beckman decomposition is a chemical property requisite to effective reactivation of phosphonylated acetylcholinesterase.

Systematic comparative studies of the NMR and UV absorption spectra of 2-PAM, 4-PAM, and 3-F-4-PAM were carried out during this year to help build a data base for (a) eventual interpretation of the comparative behaviors of these reactivators, and (b) eventual correlation between spectral behavior (e.g., oxime (aldehydic) proton chemical shift in the NMR spectrum) and reactivator behavior.

Finally during this year chemical synthesis work was initiated towards other F-substituted PAM's of interest, namely towards 4-F-3-PAM and 3-F-2-PAM. We found that in the case of 4-F-3-PAM placement of an F atom in the pyridine 4-position is not practicable because at that position of the ring the Schiemann reaction is not effective. By contrast, as our synthesis of 3-F-4-PAM has shown, at the 3-position the Schiemann reaction functions excellently. At the end of this year's research we had not yet reached the Schiemann step in the synthesis of 3-F-2-PAM but the prognosis for this modification of 2-PAM is quite favorable.

Section I

First Quarter: September 1 – November 30, 1981

Work performed in this quarter was aimed at synthesizing 3–Fluoro–4–Pyridinecarboxaldehyde oxime methiodide, the leading isomer of a family of proposed F-PAM's to be synthesized under this contract.

Two hundred grams of the commercial starting material, 2–Amino–4–Picoline, was divided into four 50-gram portions and, using the method of Roe and Seligman,[1] each was converted to a batch of 2–Hydroxy–3–Nitro–4–Picoline and 2–Hydroxy–5–Nitro–4–Picoline.

2–Amino–4–Picoline

isomeric mixture

m.p. 148–160°C
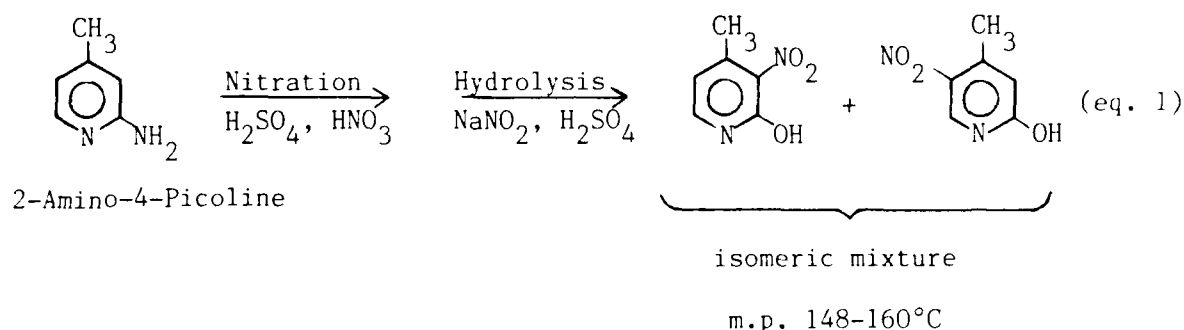
The isomers crystallized as a mixture from water, and the weights of the four batches were 33.2, 31.1, 34.3 and 36.8 g, corresponding to an average yield of 48%.

Subsequently, two of these batches were converted to the corresponding 2–Chloro–3–Nitro–4–Picoline and 2–Chloro–5–Nitro–4–Picoline by treatment with $POCl_3$.

isomeric mixture

isomeric mixture

b.p. 79–81 (0.25 torr)
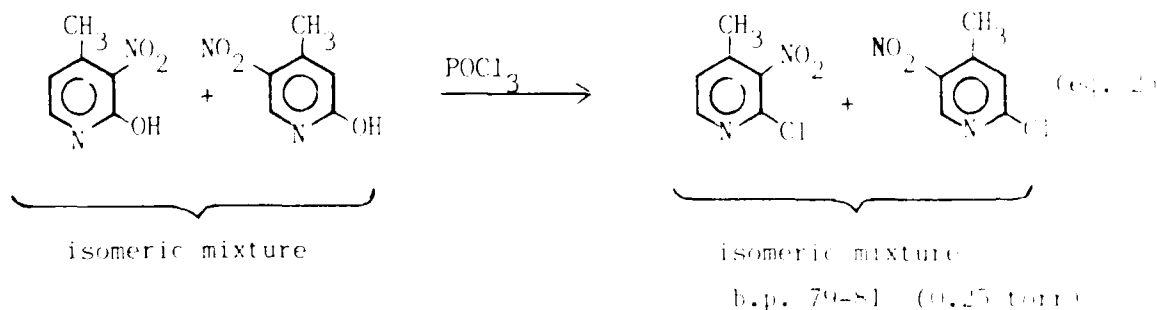
The chlorinated isomers distilled as a mixture in amounts of 24.8 g and 26.4 g for the two batches, corresponding to an average yield of 74%.

These two batches in turn were reduced to 3-Amino-4-Picoline using a Parr hydrogenation apparatus and Pd(C) catalyst.

3-Amino-4-Picoline   (eq. 3)
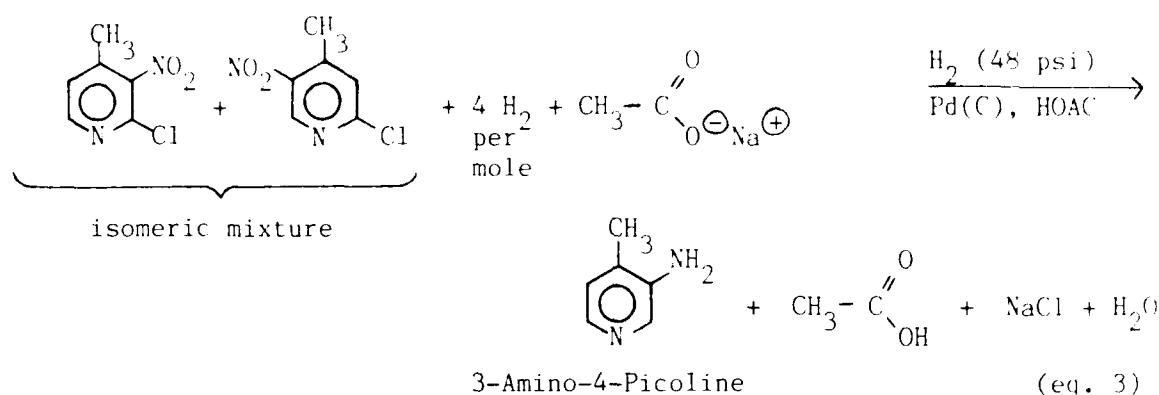
The method was essentially that of Roe and Seligman (op. cit.), except that sodium acetate was employed as a buffer in amounts equivalent with the Cl groups to be displaced.[2] The 3-Amino-4-Picoline crystallized from Et_2O, and had m.p. 103 - 106° (lit. m.p. 104 - 105°, Roe and Seligman, op. cit.). The two batches gave 11.1 and 13.5 g (71% and 82% yields respectively).

One of these batches of 3-Amino-4-Picoline (11.1 g) was then converted to 3-Fluoro-4-Picoline by the Schiemann reaction.

3-Amino-4-Picoline   Diazonium   3-Fluoro-
                              Borofluoride salt   4-Picoline

                                                          (eq. 4)
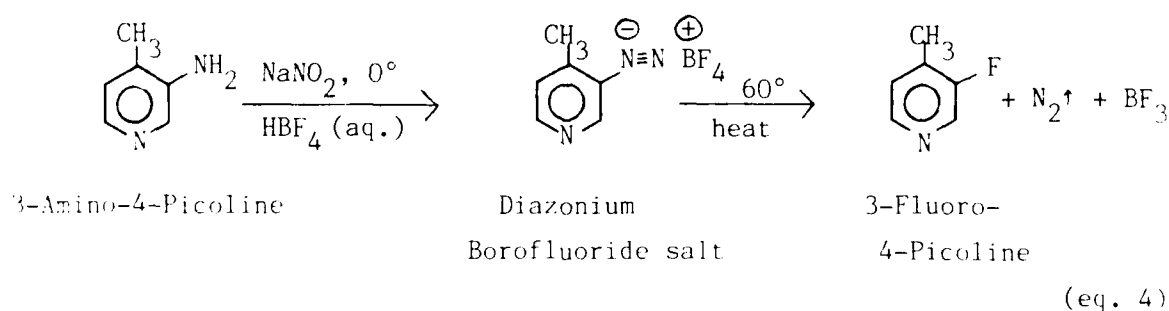
The method employed was that of Rowbotham and Schaefer[3] which uses $NaNO_2$ as the diazotization agent. Although the yield obtained by Rowbotham and Schaefer was only specifed as "good" their method was chosen in preference

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to that of Roe and Seligman since the latter uses a less convenient diazotization agent, ethyl nitrite gas.

In our hands, using $NaNO_2$, we obtained 1.33 g (12% yield) of 3-Fluoro-4-Picoline. The product was first steam-distilled and then vacuum distilled (b.p. 110° at ca. 700 torr; lit. b.p. 135° at 748 torr) and gave a picrate having m.p. 130 – 131° corresponding to that of authentic 3-Fluoro-4-Picoline (lit. m.p. 129 – 130°, Roe and Seligman, op. cit.).

Using the above product, a small scale trial experiment was carried out to determine whether the general method of Markovac, Stevens, Ash and Hackley[4] can be employed to convert 3-Fluoro-4-Picoline to 3-Fluoro-4-Pyridinecarboxaldehyde. This method is known to be effective in converting methyl groups located in the 2- and 4- positions on pyridine to aldehyde groups, and is desirable because it does so directly in one step. In the same experiment the product aldehyde can be conveniently reacted with hydroxylamine to give the corresponding oxime, which is usually crystalline.

(eq. 5)
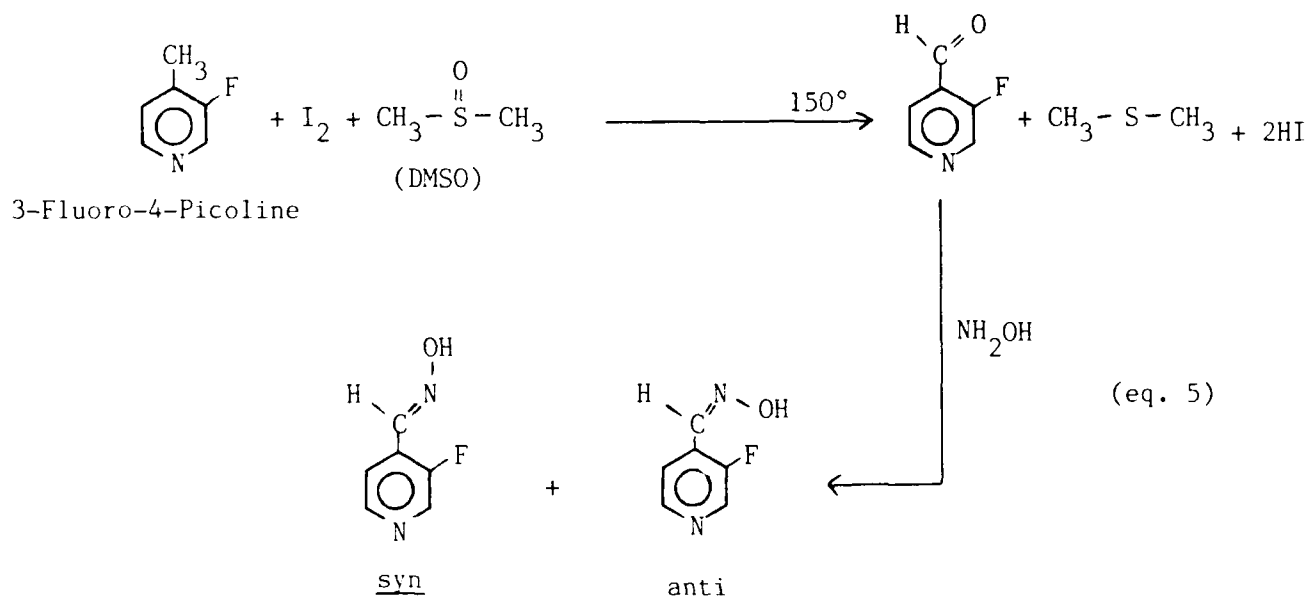
The reaction was carried out on 1.11 g of 3-FLuoro-4-Picoline and its behavior conformed exactly to the general observations of Markovac et al. A solid addition compound formed upon mixture of the picoline and I_2, and after

dissolving this compound in DMSO a vigorous reaction ensued upon heating to 150°. After working up the reaction mixture, which gave a positive dinitrophenyl-hydrazone test for aldehyde, addition of neutral aqueous NH_2OH gave a crystalline precipitate (0.397 g, 28% yield, m.p. 120 - 125°) which was recrystallized from water (0.209 g, m.p. 124 - 128°). The yield of the various oximes in the study of Markovac et al. was typically in the range of 30 - 40%.

The above experiment is situated at the cut-off point of work comprising the first quarter, and it represents only the initial stage of experiments necessary to establish genuine syn- and anti--3-FLuoro-4-Pyridinecarboxaldehyde oxime methiodide.

The following discussion will serve to summarize what has been accomplished and to designate important work for the second quarter. The first three steps (eqs. 1-3) in the preparation of 3-Fluoro-4-Picoline are now pragmatic in this laboratory and give reliable, acceptable yields. However the fourth step (eq. 4) gave us a low yield using $NaNO_2$ as the diazotization agent and in the second quarter ethyl nitrite will be employed instead. Roe and Seligman (op. cit.), whose work we have found accurate, report a 68% yield under those conditions. As can be construed from our management of materials, we have ready back-up amounts of 3-Amino-4-Picoline for input into the diazotization step.

The results obtained in the final experiment (eq. 5) strongly suggest that the method of Markovac et al. is applicable to 3-Fluoro-4-Picoline, to convert it directly to 3-Fluoro-4-Pyridinecarboxaldehyde oxime. Early in the second quarter the crystalline product described above will be analyzed by nmr, ir, and if such spectral analysis supports the 3-Fluoro-4-Pyridine carboxaldehyde oxime structure, elemental analysis. Additionally, if judged necessary, the compound will be scrutinized by mass spectroscopy.

Contingent upon rigorous structure proof, the Markovac-Stevens-Ash-Hackley reaction will be scaled up and at the same time attention will be paid to details of oxime geometrical isomerism. As seen in eq. 5, both syn- and anti-isomers are possible, and one or the other may predominate. The oximation product mixture will be analyzed by GC, HPLC and nmr, together with possible fractional crystal-lization , and conditions of oximation (e.g. temperature, pH) may be varied to increase formation of the minor isomer. Short of x-ray crystallography, assignment of geometric structure is best made by comparative nmr spectroscopy, requiring both

isomers, as exemplified by the study of Poziomek, Kramer, Mosher and Michel.[5]

In addition to the above synthetic and physical determinations, the syn- and anti-3-Fluoro-4-Pyridinecarboxaldehyde oximes will be converted to their corresponding methiodides, which are the desired 3-F-4-PAM's. These compounds will be physically characterized, including determination of their pKa values.

It is projected additionally that in the second quarter synthetic work toward the 3-F-2-PAM's will be initiated.

Section II

Second Quarter: December 1, 1981 - February 28, 1982

Work performed in this quarter achieved (1) the final steps of the synthesis, in gram amounts, of 3-Fluoro-4-Pyridinecarboxaldehyde oxime methiodide (3-F-4-PAM), and (2) determination of some of the physical properties of this compound relevant to future studies regarding potency to reactivate phosphonylated acetylcholinesterase.

In the Section I, First Quarter, a poor yield (12%) of the synthetic precursor 3-Fluoro-4-Picoline was obtained from the Schiemann reaction. That situation has been significantly improved by employing ethyl nitrite (EtONO) instead of sodium nitrite as the diazotization agent.[1] A suitable method for EtONO generation is given in <u>Organic Syntheses</u>.[6]

(eq. 1)

3-Amino-4-Picoline	Diazonium	3-Fluoro-4-Picoline
13.5 g	Borofluoride salt	8.50 g distilled
(0.125 Mole)		(0.076 Mole)
		61% yield
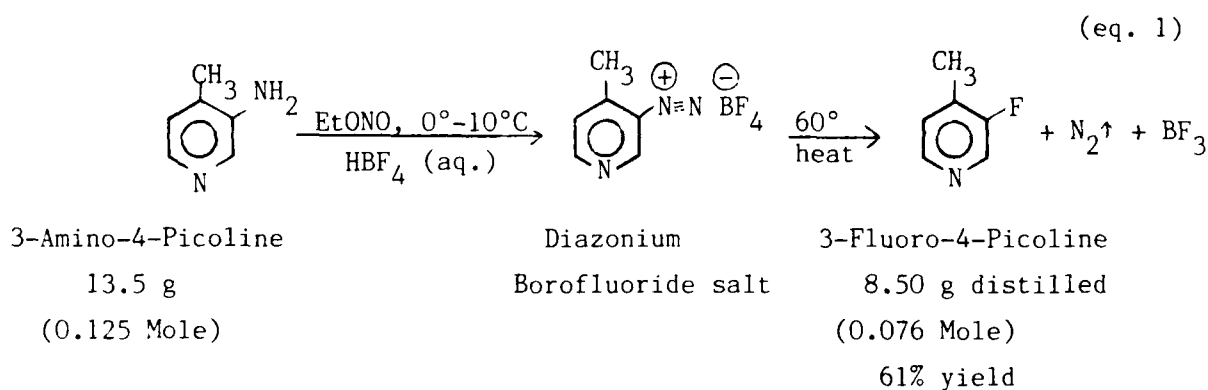
Since a preliminary small-scale experiment in the first quarter indicated that 3-Fluoro-4-Picoline can be converted directly to 3-Fluoro-4-Pyridinecarboxaldehyde oxime by the general method of Markovac, Stevens, Ash and Hackley,[4] we scaled this reaction up, using 8.50 g of starting material.

$$\text{3-Fluoro-4-Picoline} + I_2 + CH_3-\overset{O}{\underset{||}{S}}-CH_3 \xrightarrow{140°} \text{product} + CH_3-S-CH_3 + 2HI \quad (eq.\ 2)$$

3-Fluoro-4-Picoline
8.50 g
(0.076 Mole)

(DMSO)

2M NH_2OH
pH 7.4
room temp.

syn   anti
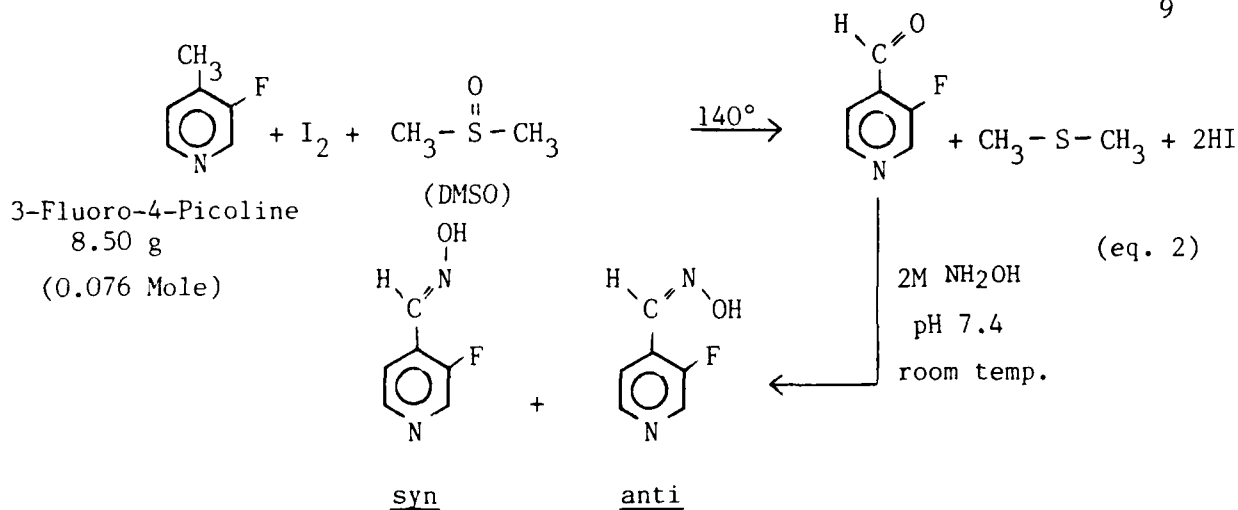
Work-up gave a crude product mixture weighing 4.05 g, a sample of which was examined by analytical-scale HPLC using a reverse phase column (H_2O/MeOH solvent system) on our beckman-Altex instrument. The chromatograms (see Figs. 1a and 1b) indicated two components comprising about 96% and 4%, which may correspond to two geometrical isomers depicted in eq. 2. The pyridinecarboxaldehyde oximes can be detected in nMole amounts due to their strong absorption in the UV (254 nm).

Careful crystallization from H_2O/EtOH gave 3.15 g of the major component (which we will call isomer A, m.p. 125 – 128°C) contaminated with about 1.5% of what we assume to be isomer B (see Fig. 2a). The supernatant material from the crystallization was mostly A but enriched to about 6.5% in B (see Fig. 2b) which to date we have not been able to isolate. In principle this could be achieved by preparative-scale HPLC but has not yet been attempted.

For purposes of comparison we have examined the HPLC behavior of a commercial sample of 4-Pyridinecarboxaldehyde oxime (Sigma Chemical Co.) See Fig. 3a) and co-chromatographed it with our 3-F analog (Fig. 3b). The two compounds have similar retention times, but while the 3-F compound gives a sharp elution peak the non-Fluoro compound gives a broad peak for reasons presently not clear to us.

It is tempting to assign the syn-configuration to our component A, in simple analogy to the non-Fluoro case reported by Poziomek, Kramer, Mosher and Michel.[5] These workers found the syn isomer to greatly predominate over the anti such that they could isolate the latter only in yields less than 5%. However in our case such an assignment would be premature and should await additional studies.

Based on the weight of our twice-crystallized component A (2.90 g), the yield for eq. 2 is at least 27%, which is comparable to the yields obtained for the reactions studied by Markovac, et al. (op. cit.).

That component A is structurally 3-Fluoro-4-Pyridinecarboxaldehyde oxime (MW 140.12) is supported by its mass spectrum (Fig. 4) and its proton NMR spectrum (Fig. 5).
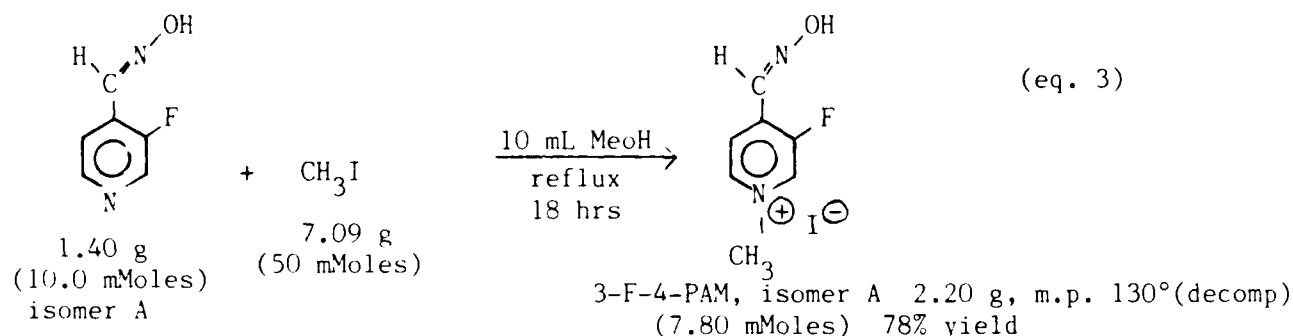
In the mass spectrum the base peak is at m/e 122 corresponding to water loss from the molecular ion. As expected, the molecular ion (M+) peak at m/e 140 is present and in nearly the same intensity as the water loss peak. Additionally there is an (M+1) peak (m/e 141) of about 7% the intensity of the molecular ion peak, as is expected for a molecule of $C_6H_5N_2OF$ constituency (there is no F isotope to contribute to an M+1 population).

In the proton NMR spectrum (Fig. 5), obtained in D_2O solvent with a 3-(Trimethylsilyl) propane sulfonic acid sodium salt (DSS) as internal standard, all of the resonance peaks occur downfield from DSS at chemical shifts greater than 7.50 ppm. Interpretation is best made in comparison with the corresponding spectrum of the non-Fluoro analog, 4-Pyridinecarboxaldehyde oxime (Sigma Chemical Co.) shown in Fig. 6. In the latter the four protons on the pyridine ring appear as a pair of internally split doublets, one doublet centered near 7.60 ppm and the other near 8.50 ppm. The doublet located most downfield (8.50 ppm) corresponds to the two protons (H-2 and H-6) adjacent to the nitrogen in the pyridine ring. The other doublet corresponds to the two protons (H-3 and H-5) adjacent to the carboxaldehyde oxime group. Integrations of the respective resonances in the spectrum are proportional to the number of protons participating. The acidic proton on the oxygen of the oxime group is washed out in D_2O and doesn't appear. In these spectra an upfield peak due to hydroxyl impurity in the D_2O solvent was omitted for cosmetic reasons.

In the spectrum of the 3-F compound (Fig. 5), the pattern of resonances is changed relative to Fig. 6 because the F atom on the pyridine ring renders the protons more nonequivalent. The spectrum is additionally complicated in that substitution of F for H in the 3-position results in a shifting (deshielding) of the aldehydic proton resonance downfield so as to coincidentally overlap with the resonance of one of the ring protons (H-6) at 8.40 ppm. The narrow doublet at slightly lower field (centered near 8.50 ppm) is due to H-2 split by the 3-F atom. It (H-2) is the most deshielded proton in this compound since

it is situated between the pyridine nitrogen and the F atom. The conspicuous triplet centering at 7.72 ppm is interpreted as due to H-5, which by contrast is the least deshielded of the protons. It is a triplet because it is split by both the 3-F and the H-6 atoms with similar coupling constants. It is known from studies of Fluoro Picolines[3] that the coupling constants between ortho-related protons and between meta-related F and H atoms in pyridine compounds can be of similar magnitudes. The proton on the oxygen of the oxime group is washed out in D_2O solvent.

The final reaction in the synthesis of 3-Fluoro-4-Pyridinecarboxaldehyde oxime methiodide (3F-4-PAM) is quaternization of the pyridine group in 3-F-4-Pyridinecarboxaldehyde oxime with methyl iodide. This was achieved using excess methyl iodide in refluxing MeOH (eq. 3).

(eq. 3)

1.40 g
(10.0 mMoles)
isomer A

+ CH_3I

7.09 g
(50 mMoles)

10 mL MeOH
reflux
18 hrs

3-F-4-PAM, isomer A   2.20 g, m.p. 130°(decomp)
(7.80 mMoles)   78% yield

The reaction is not fast and requires about 18 hrs. The product 3-F-4-PAM is designated as isomer A since it is reasonable to assume that during the pyridine quatrnization the geometrical configuration of the oxime group is preserved, as was found by Poziomek et al. (op. cit.). The 3-F-4-PAM is quite soluble in water and is crystallizable from ethanol. It develops clumps of orange crystals which when pulverized give a rich yellow color.

A proton NMR spectrum of this material is given in Fig. 7. There is some broadening of the resonances, and relative to the unquaternized precursor (Fig. 5) the pyridine ring protons are significantly shifted downfield due to the deshielding effect of the positive charge. The resonance due to the aldehydic proton is much less affected (at 8.45 ppm) and it no longer overlaps with the doublet of H-6 (which has been shifted downfield) but now overlaps with the triplet of H-5 which was previously upfield. A new peak in the spectrum, ie. the strong singlet at 4.37 ppm, is due to the methyl group bonded to the pyridine nitrogen. This methyl group is significantly downfield from the internal standard due to the considerable deshielding effect of the positively

charged nitrogen. Included in this spectrum is the resonance due to the hydroxyl impurity in the D_2O solvent plus some small peaks due to residual solvent of crystallization (EtOH). For purposes of gross comparison an NMR spectrum of commercial 2-PAM (Sigma Chemical Co.) is included (Fig. 8) but will not be interpreted in this report.
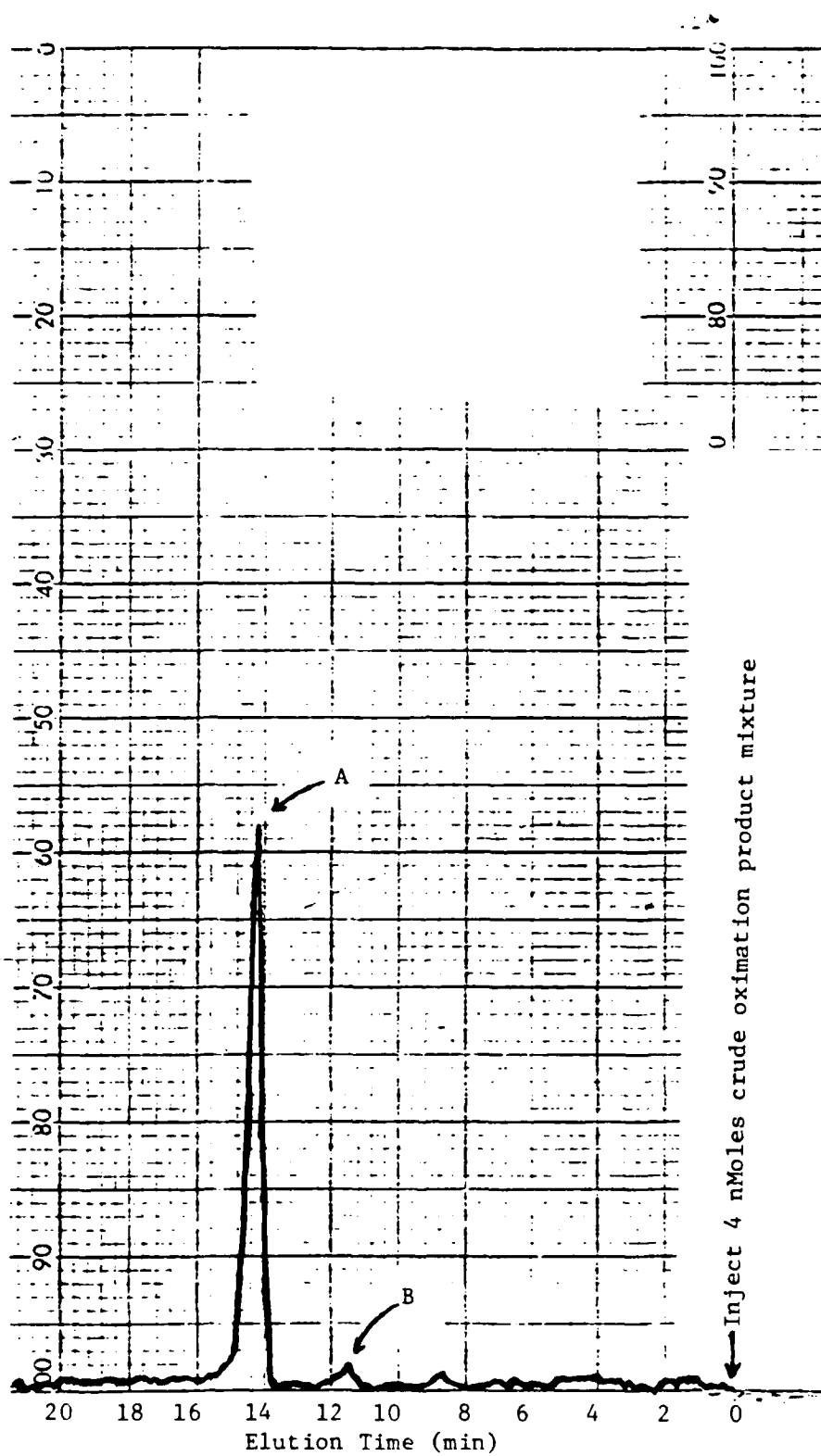
Samples of both 3-F-4-Pyridinecarboxaldehyde oxime and 3-F-4-PAM have been sent (Feb. 18) to Schwarzkopf Microanalytical Laboratory, Woodside, NY, for elemental microanalysis, but at the time of this report the analyses had not yet been performed. Results are reported in Addendum, p. 25.

Finally in this quarter we determined the pK_a (in doubly-distilled degassed H_2O at 25 - 27°C) of 3-F-4-PAM isomer A and, for reference purposes, 2-PAM (Sigma Chemical Co.). For 3-F-4-PAM we found pK_a 8.18 $\pm$ .01 and for 2-PAM we found pK_a 8.01 $\pm$ .01. The determinations were made by titrating replicate sample solutions of the PAM compound (0.50 m Mole in 25 mL H_2O) with standard 0.10 N NaOH and concurrently measuring the pH at various points in the titration with our research pH meter.

These pK_a values occasion a brief annotation regarding the pK_a values reported previously in the literature for PAM compounds. Our original proposal ("Construction of New Potentia. Reactivators of Phosphonylated Acetylcholinesterase: Substitution of F for H in the Nucleus of Pyridinecarboxaldehyde Oximes") adopted for purposes of argumentation the pK_a values reported by Hagedorn, Stark and Lorenz.[7] They list pK_a 7.68 for 2-PAM and pK_a 8.34 for 4-PAM. However these values are at variance with those originally reported by Ginsburg and Wilson,[8] namely 8.0 for 2-PAM and 8.6 for 4-PAM. In view of the close agreement between our value for 2-PAM (8.01) and that of Ginsburg and Wilson (8.0), we wish to utilize th2 pK_a values generally reported by Ginsburg and Wilson as points of reference in subsequent discussions. Taking their value of pK_a 8.6 for 4-PAM as accurate, substitution of F for H in the 3-position of the pyridine nucleus of 4-PAM lowers the pK_a from 8.6 to 8.18, about 0.4 pK_a units. Resultantly 3-F-4-PAM is in the optimal pK_a range for reactivation of phosphonylated acetylcholinesterase. Concerning the concept of optimal pK_a range see Hagedorn, Stark and Lorenz (op. cit.).

In this connection it is relevant to note that the pK_a of 3-F-4-PAM essentially coincides with that of the known reactivator TMB-4, pK_a 8.2 (refs. 9 and 10). The TMB-4 equals or surpasses the potency of 2-PAM as a reactivator of poisoned acetylcholinesterase, and therefore it will be of considerable theoretical and practical interest to determine the comparative effectivity of the structurally simpler 3-F-4-PAM molecule.

13

In Section III additional quantities of 3-F-4-PAM will be prepared from our supply of synthetic precursors, further attempts will be made to obtain and examine isomer B, and synthetic reactions will be carried out toward generating 3-F-2-PAM, another new compound designated in this contract.

HPLC chromatogram of crude oximation product mixture (4 nMoles)

Fig. 1a
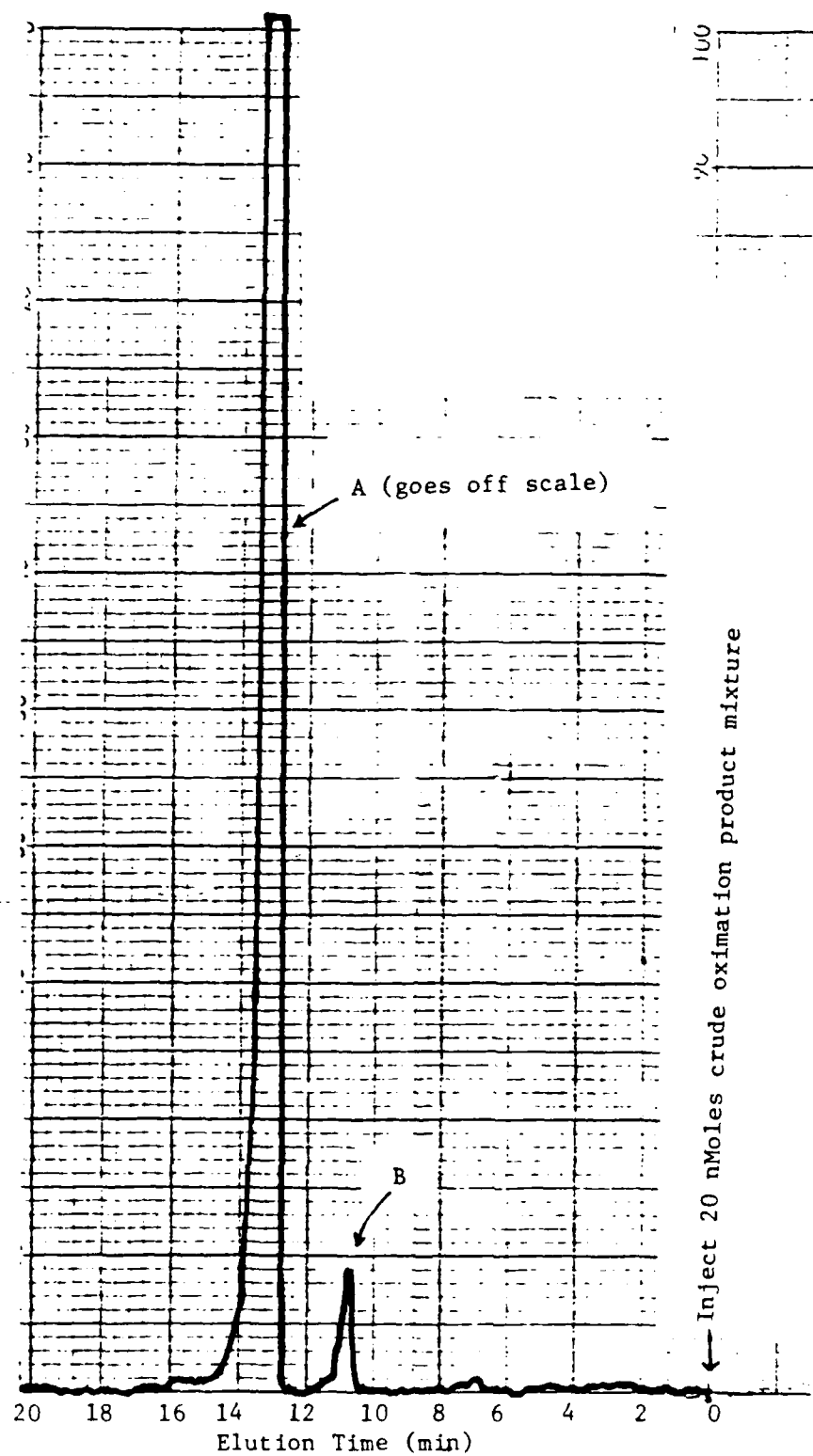
HPLC chromatogram of crude oximation product mixture (20 nMoles)

Fig. 1b
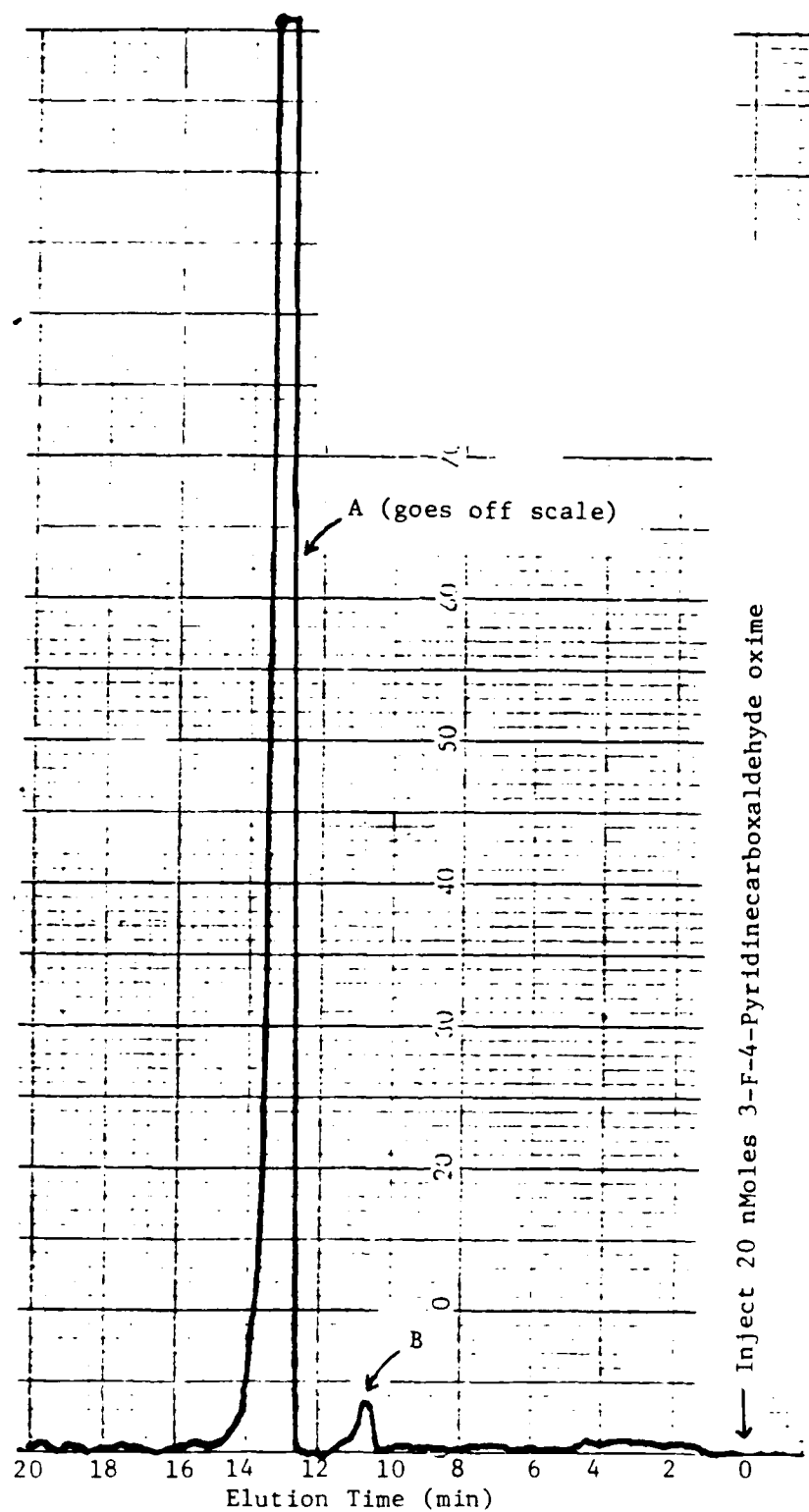
HPLC chromatogram of crystallized oximation product (20 nMoles)
(3-F-4-Pyridinecarboxaldehyde oxime)

Fig. 2a
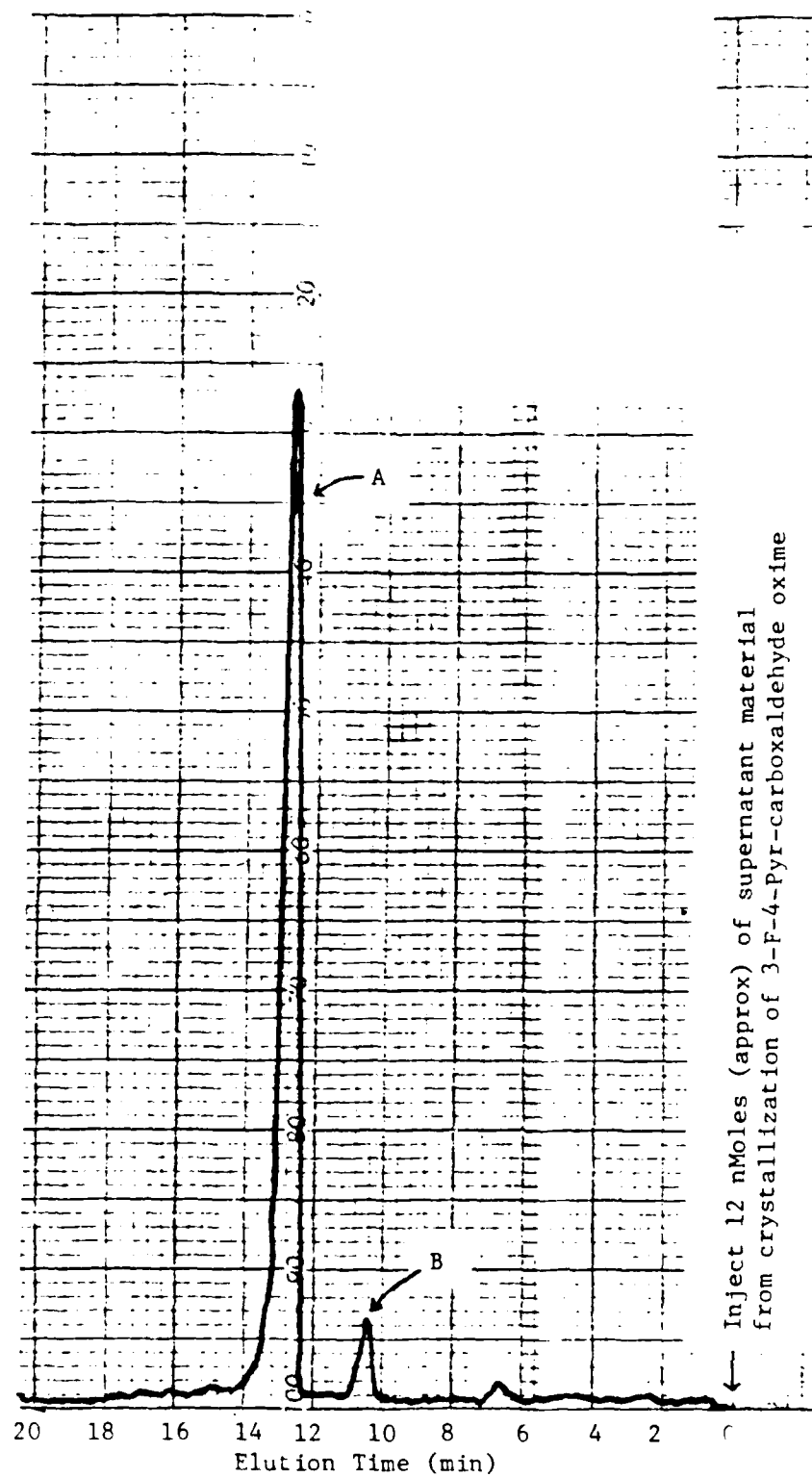
HPLC chromatogram of supernatant from oxime crystallization

Fig. 2b
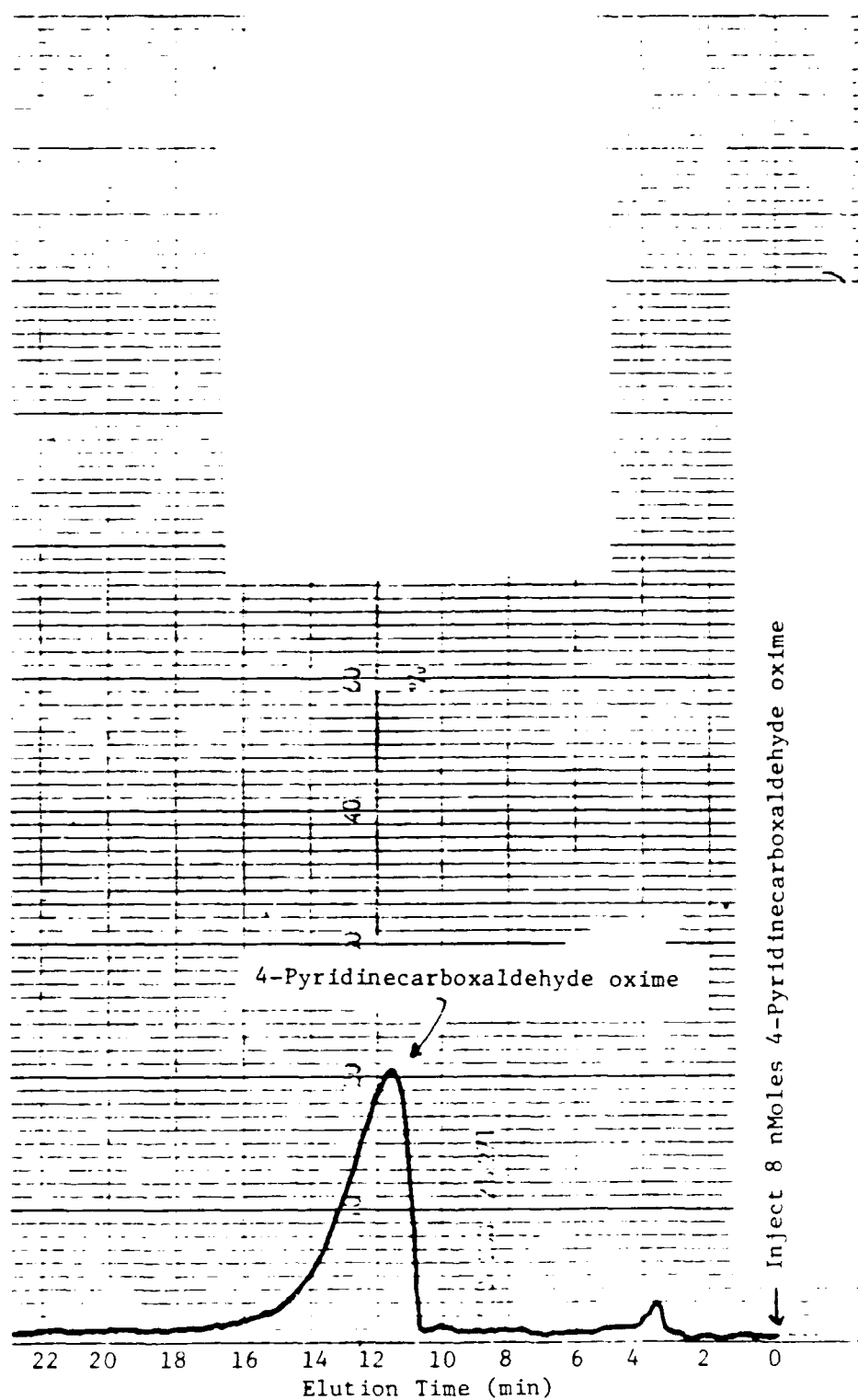
HPLC chromatogram of 4-Pyridinecarboxaldehyde oxime

Fig. 3a
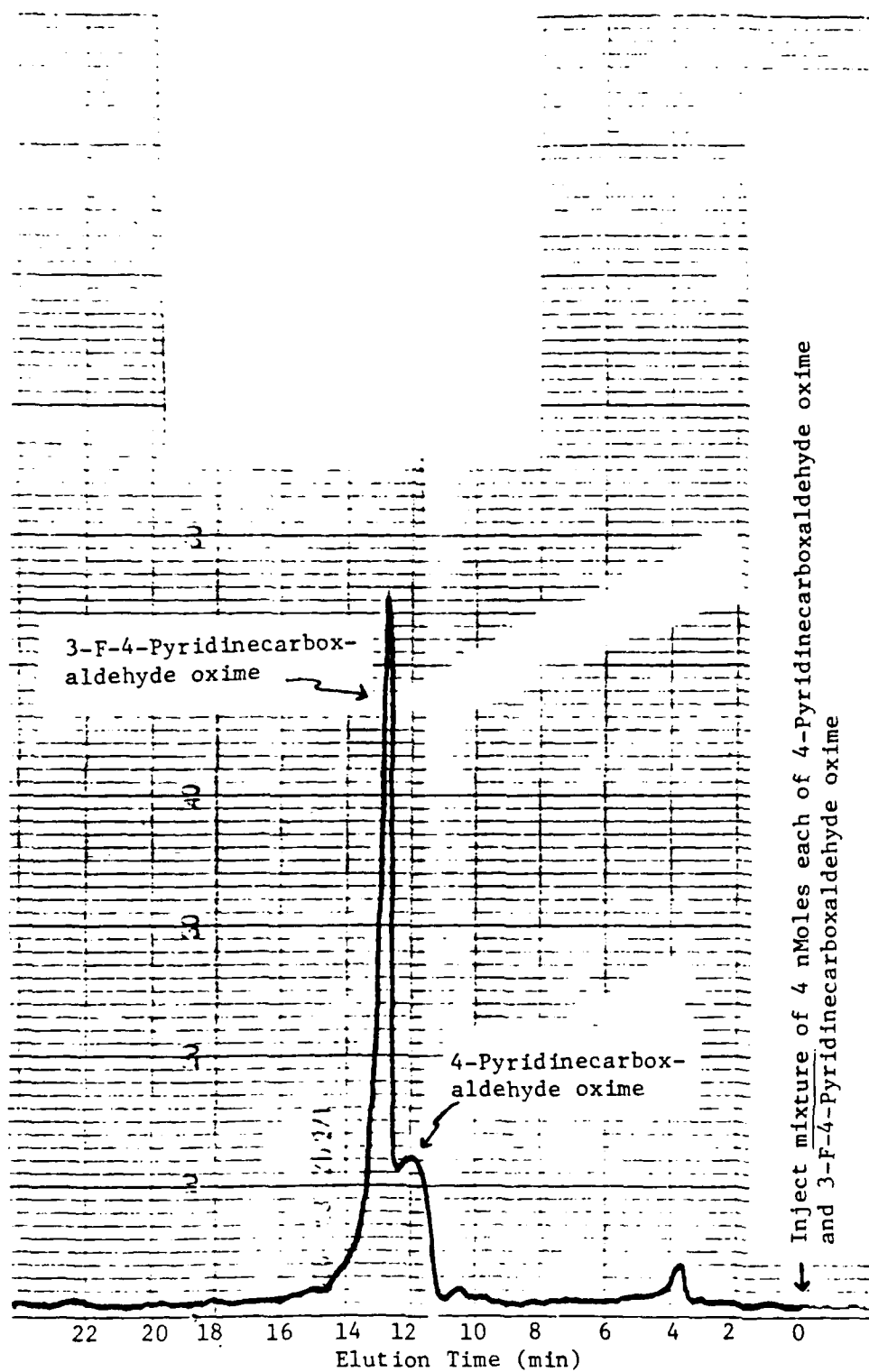
HPLC chromatogram of a mixture of 4-Pyridinecarboxaldehyde oxime and 3-F-4-Pyridinecarboxaldehyde oxime

Fig. 3b
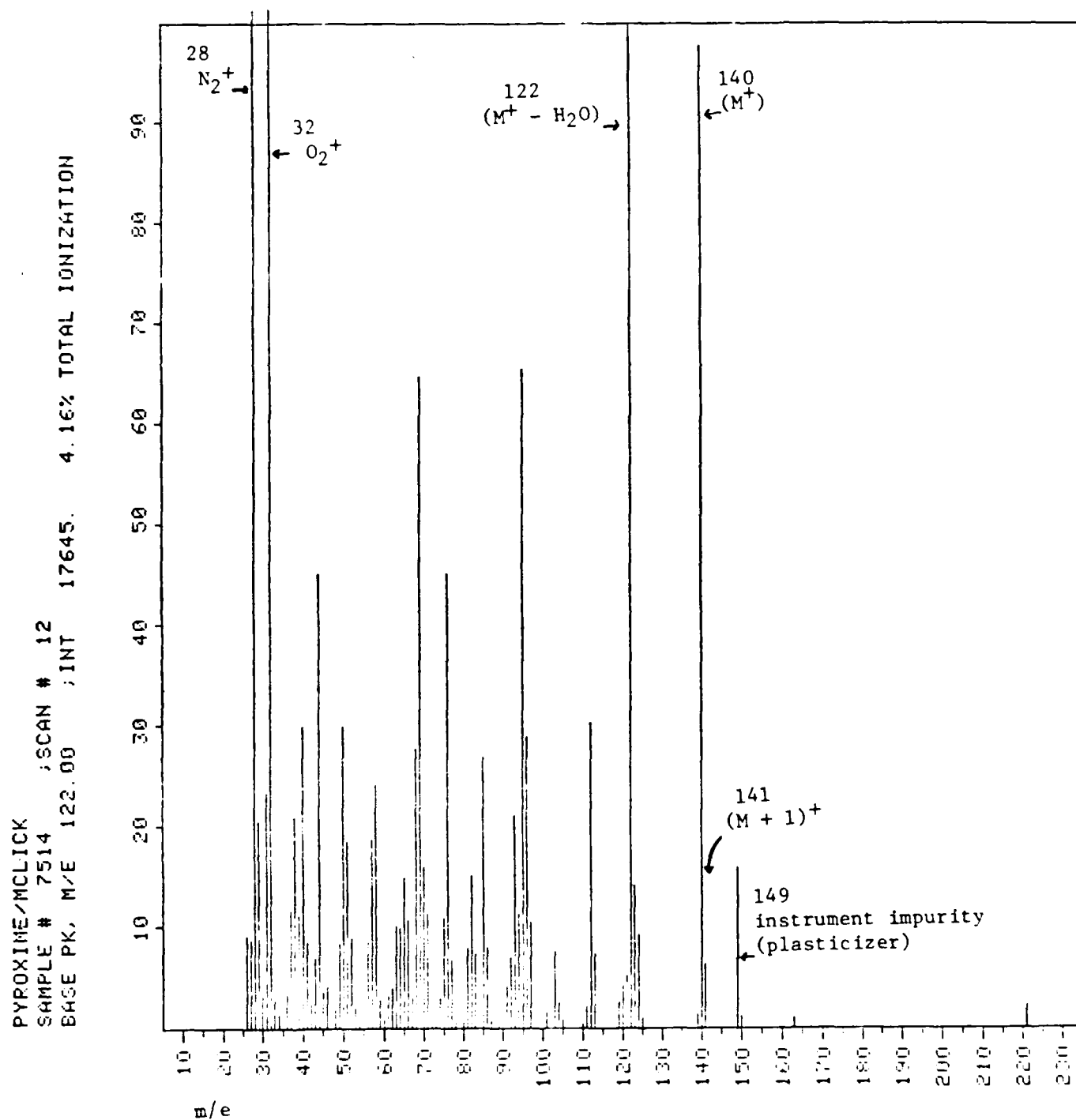
PYROXIME/MCLICK
SAMPLE # 7514   ;SCAN # 12
BASE PK, M/E 122.00   ;INT   17645.   4.16% TOTAL IONIZATION

Mass spectrum of 3-Fluoro-4-Pyridinecarboxaldehyde oxime

Fig. 4

Proton NMR spectrum of 3-Fluoro-4-Pyridinecarboxaldehyde oxime
in deuterium oxide solvent

Fig. 5

FT-80A SPECTRUM NO. _F-Y_

OPERATOR _I. Mc kick_ DATE _11/9/81_

NUCLEUS _'H_ FREQUENCY _11/9/81_

SYNTHESIZER SETTING _____

EXPERIMENT NAME _____

FILE NAME _____

SAMPLE _(4)_

LOCK ☑INTERNAL ☐EXTERNAL

LOCK SIGNAL _D₂O_ TEMP _26_ °C

SPIN RATE _6_ rps. _____ mm

INSERT _8_ mm

ACQUISITION

SPECTRAL WIDTH (SW) _1000_ Hz

NO. OF TRANSIENTS (NT) _42_

ACQUISITION TIME (AT) _3_ sec.

PULSE WIDTH (PW) _45_ μsec.
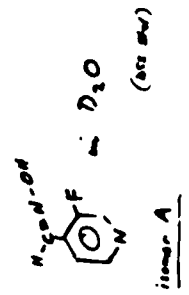
PULSE DELAY (PD) _0_ sec.

DATA POINTS (DP) _6000_

TRANSMITTER OFFSET (TO) _96_

HIGH FIELD _/_ LOW FIELD ____

RECEIVER GAIN (RG) _2_

DECOUPLER MODE (DM) _____

DECOUPLER OFFSET (DO) _____

NOISE BANDWIDTH (NB) _____ kHz

ACQUISITION MODE (AM) _____

DISPLAY

SENS. ENHANCEMENT (SE) _0_ sec.

WIDTH OF PLOT (WP) _552_ Hz

END OF PLOT (EP) _.50_ Hz

WIDTH OF CHART (WC) _100_ Hz

END OF CHART (EC) _0_ Hz

VERTICAL SCALE (VS) _192_

REFERENCE LINE (RL) _0.00_ (DSS)

ppm
chemical
shift

VARIAN

22

1	8.60	684.3	26
2	8.58	682.5	17
3	8.54	678.6	35
4	8.52	678.0	42
5	8.23	655.20	43
6	7.64	608.0	19
7	7.62	606.7	26
8	7.59	603.7	15
9	7.58	603.4	23
10	7.56	602.0	32
11	7.56	601.7	40
12	0.00	0.0	18

DSS

solvent
hydroxyl impurities

8.23 ppm
aldehydic proton

Proton NMR spectrum of 4-Pyridinecarboxaldehyde oxime
in deuterium oxide solvent

Fig. 6

¹H

CFT-20 SPECTRUM NO. *J-7*

OPERATOR *J. McLick* DATE *6/11/74*

SAMPLE *3-F-4-PAM (Isomer A)*

isomer A
—————
3-F-4-PAM

DSS int. std.

LOCK SIGNAL *D₂O*

SPIN RATE *6* rps.   TEMP *est.°C*

ACQUISITION

SPECTRAL WIDTH (SW) *1000* Hz

NO. OF TRANSIENTS (NT) *10*

ACQUISITION TIME (AT) *3* sec.

PULSE WIDTH (PW) *25* µsec.

PULSE DELAY (PD) *0* sec.

DATA POINTS (DP) *6000*

TRANSMITTER OFFSET (TO) *%*

HIGH FIELD. ✓ LOW FIELD.

RECEIVER GAIN (RG) *8*

DECOUPLER MODE (DM)

DECOUPLER OFFSET (DO)

NOISE BANDWIDTH (NB) _____ kHz

DISPLAY

SENS. ENHANCEMENT (SE) *0* sec.

WIDTH OF PLOT (WP) *850* Hz

END OF PLOT (EP) *-50* Hz

WIDTH OF CHART (WC) *900* Hz

END OF CHART (EC) *0* Hz

VERTICAL SCALE (VS) *104*

REFERENCE LINE (RL) *0.00 (DSS)*

varian

0 ppm
chemical
shift

DSS

EtOH
residual solvent

EtOH
residual solvent

EtOH
residual solvent

4.70 ppm hydroxyl impurity in solvent →

8.45 ppm aldehydic proton

Proton NMR spectrum of 3-F-4-PAM in deuterium oxide solvent

Fig. 7

^1H

CFT-20 SPECTRUM NO. T-8
OPERATOR J.M.Lick DATE 4/11/73

SAMPLE unused 2-PAM ($^1H-?$)
2-PAM

: D_2O

2-PAM

DSS int. std.

LOCK SIGNAL D_2O
SPIN RATE 6 rps.   TEMP amb °C

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO. OF TRANSIENTS (NT) 10
ACQUISITION TIME (AT) 3 sec
PULSE WIDTH (PW) 45 sec
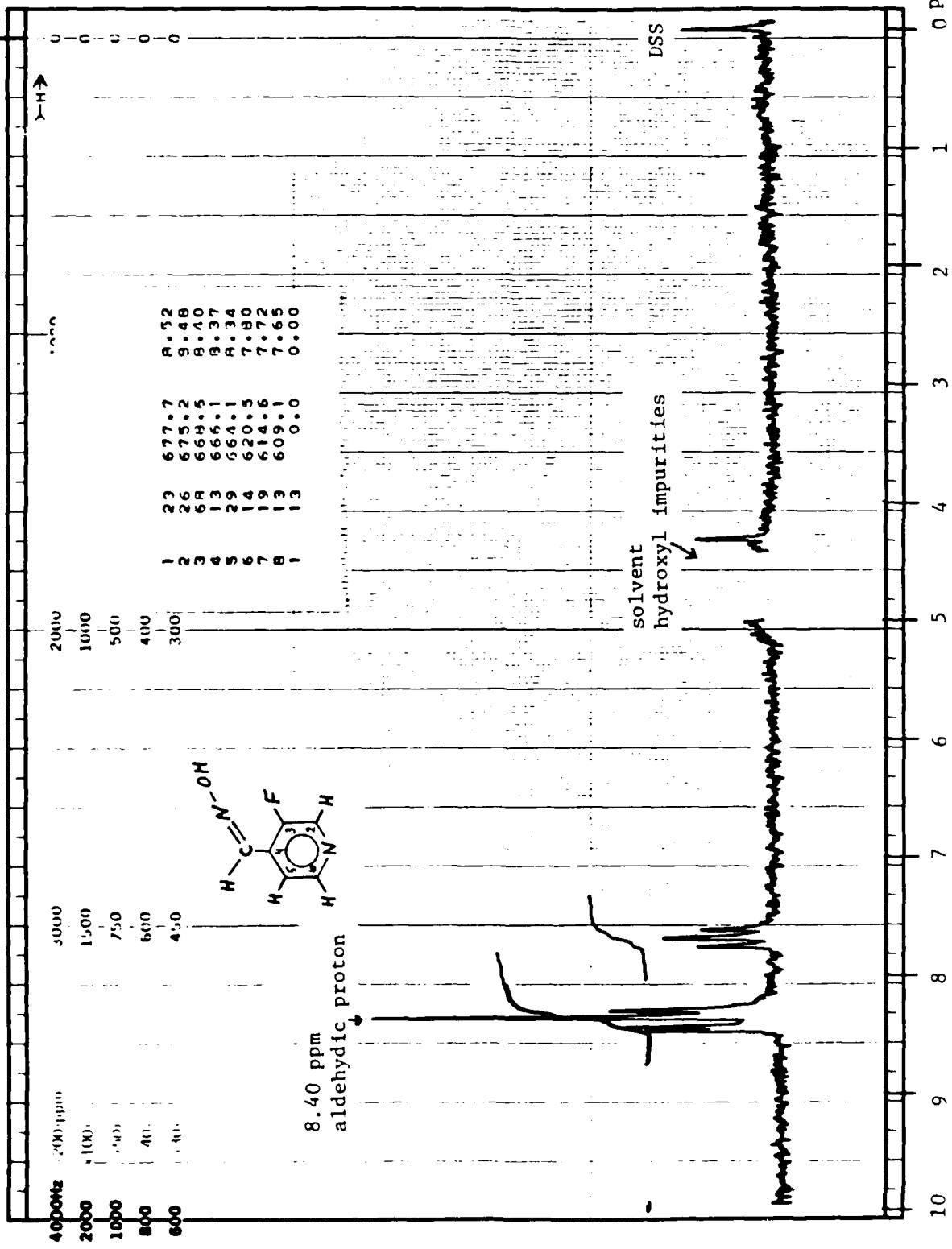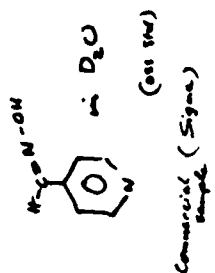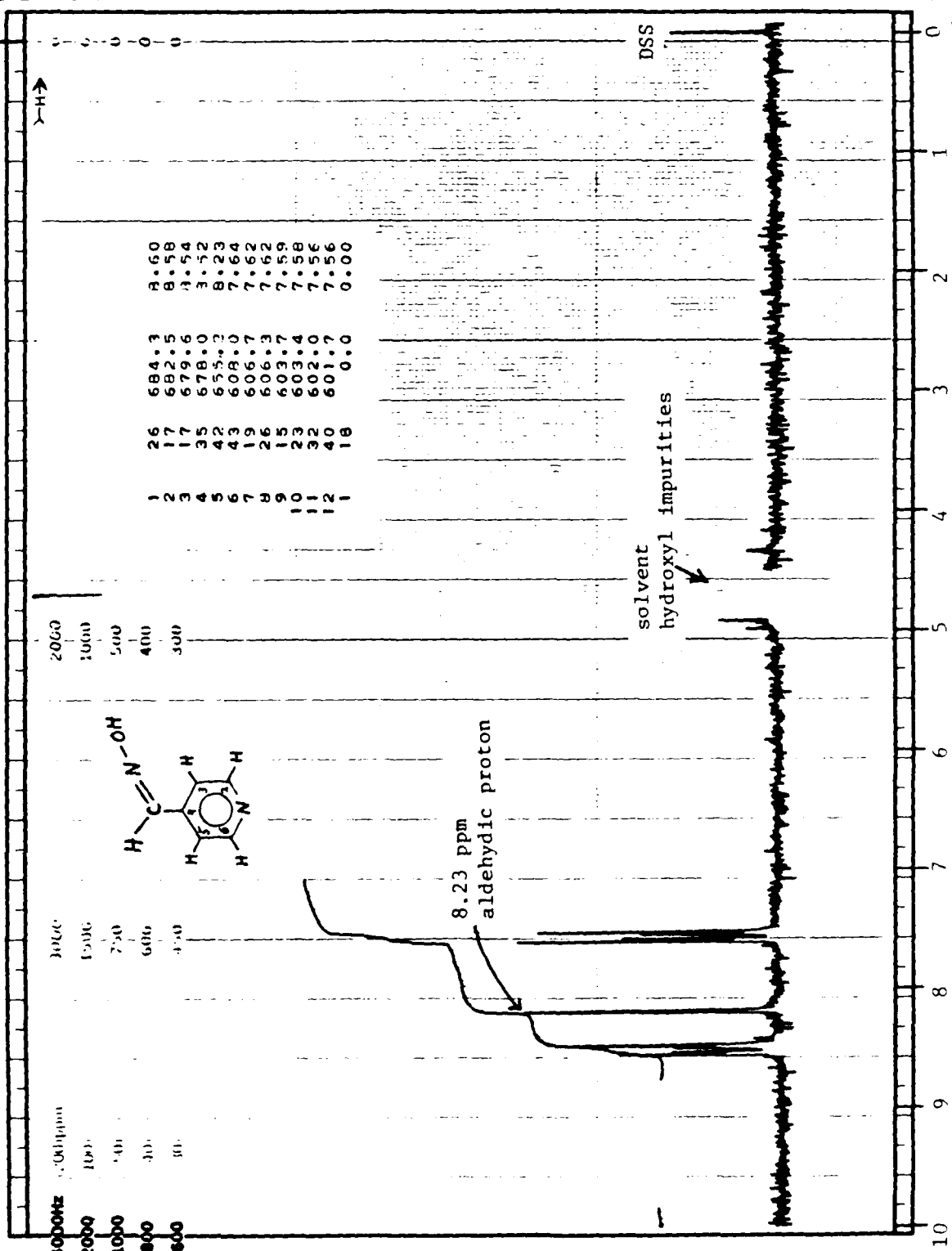PULSE DELAY (PD) 0 sec
DATA POINTS (DP) 6000

TRANSMITTER OFFSET (TO) 96
HIGH FIELD ✓   LOW FIELD
RECEIVER GAIN (RG) 2

DECOUPLER MODE (DM)
DECOUPLER OFFSET (DO)
NOISE BANDWIDTH (NB) kHz

DISPLAY
SENS. ENHANCEMENT (SE) 0 sec
WIDTH OF PLOT (WP) 150 Hz
END OF PLOT (EP) 50 Hz
WIDTH OF CHART (WC) 800 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 100
REFERENCE LINE (RL) 600 (DSS)

varian
PRINTED IN USA

chemical shift

DSS

4.70 ppm hyroxyl impurity in D_2O

Proton NMR spectrum of 2-PAM in deuterium oxide solvent

Fig. 8

ADDENDUM

ELEMENTAL ANALYSIS REPORT (SCHWARZKOPF MICROANALYTICAL LABORATORY, WOODSIDE, NY 11377):

3-F-4-PYRIDINECARBOXALDEHYDE OXIME

$C_6H_5N_2OF$

MW 140.12

	THEORY	FOUND
%C	51.43	51.54
%H	3.59	3.59
%N	20.00	20.28
%F	13.56	13.50

3-F-4-PYRIDINECARBOXALDEHYDE OXIME-
METHIODIDE

$C_7H_8N_2OFI$

MW 282.06

	THEORY	FOUND
%C	29.81	29.90
%H	2.86	3.10
%N	9.93	9.45
%F	6.74	7.03
%I	44.99	44.71

Section III
Third Quarter: March 1 – May 31, 1982

Chemical synthesis during this quarter concerned (1) work on two additional batches of precursors of 3-F-4-PAM, whose synthesis and molecular structure data were detailed in the previous report, and (2) the initial synthesis steps towards 3-F-2-PAM and 4-F-3-PAM.

For 3-F-2-PAM we started with 6-Amino-2-Picoline (Aldrich) and nitrated this compound following the method of Parker & Shive[11] modified by Baumgarten & Chien-Fan Su.[12]

6-Amino-2-Picoline 250 g → A   B (steam – volatile)
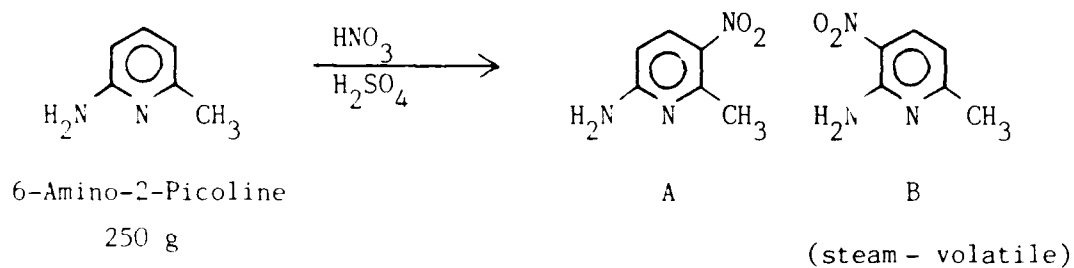
Of the two main nitration products, only isomer A is wanted. In the literature (refs. cited above), A is separated from B by steam distillation, since B is steam-volatile but A is not. However, even in relatively small batches we found this steam distillation to be so inefficient as to be

impracticable using our homemade, Bunsen-burner-powered steam generator. Therefore we are placing an order for a portable electric steam generator of suitable capacity to achieve this necessary distillation as well as others in our synthesis work.

Once isomer A is purified in sufficient quantities, it will be converted, by way of several additional steps, to 3-Fluoro-2-Picoline which should be directly transformable to 3-F-2-Pyridinecarboxaldehyde oxime by the Markovac-Stevens-Ash-Hackley reaction.

Additionally we have carried out the initial step toward to synthesis of 4-F-3-PAM, also a nitration:

3-Picoline-N-oxide   4-Nitro-3-Picoline-N-oxide

100 g (Aldrich)   m.p. 135-138°C, 57 g (40.2% yield)
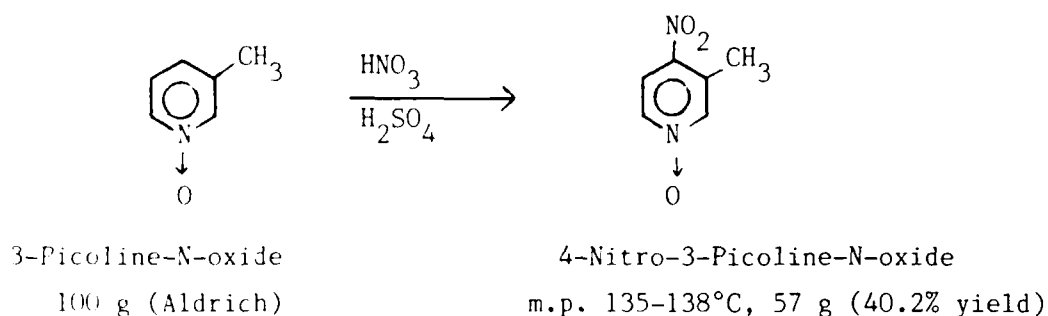
We utilized the procedure of Herz & Tsai,[13] and the desired 4-Nitro isomer is isolated by crystallization. At the time of this report we are preparing to reduce the 4-Nitro-3-Picoline-N-Oxide using the Parr hydrogenation apparatus. The reduction product, 4-Amino-3-Picoline, can in turn be converted to 4-Fluoro-3-Picoline using the Schiemann reaction.[14] Ultimate conversion to 4-Fluoro-3-Pyridinecarboxaldehyde oxime will have to be by a pathway other than the Markovac-Stevens-Ash-Hackley reaction, since the latter applies only to methyl groups in the

2- or the 4- position of the pyridine ring, not the 3- position. Therefore, we plan to use the method cited by Ginsburg & Wilson[8] for their preparation of 3-Pyridinecarboxaldehyde and its oxime, namely that of Panizzon.[15] Our assumption is that during the requisite synthetic steps the 4-Fluoro group will remain intact. Concerning physical measurements, we have repeated our pK_a determinations for 3-F-4-PAM and 2-PAM and extended these measurements to 4-PAM. In the previous quarter we obtained pK_a 8.18 for 3-F-4-PAM and pK_a 8.01 for 2-PAM. In this quarter we have obtained pK_a 8.12 for 3-F-4-PAM and pK_a 8.00 for 2-PAM. The respective values for 3-F-4-PAM and 2-PAM obtained during the two separate quarters agree well. These values were discussed with regard to prior literature values and with regard to mechanism implications in Section II. The value obtained for 4-PAM, namely pK_a 8.50, is 0.10 pK_a unit below that reported by both Ginsburg & WIlson[8] and Poziomak, Kramer, Mosher, and Michel,[5] but 0.16 pK_a units above that reported by Hagedorn, Stark and Lorenz.[7] In any event the pK_a of 4-PAM lies outside of the "pK_a optimum range" for effective reactivation, which, based on a value of 8.0 for 2-PAM, is 7.8 to 8.3.

In Section II, Second Quarter, we did not include the elemental microanalysis results for 3-F-4-Pyridinecarboxaldehyde oxime and 3-F-4-PAM since, although samples had been submitted to Schwarzkopf Microanalytical Laboratory, the analyses had not yet been performed. The results were subsequently obtained, judged satisfactory, and are included on a separate sheet labeled as an addendum in this report (page 32). Concerning the molecular geometry of the oxime groups in these compounds, samples have been submitted for X-ray crystal structure analysis but the results are not yet obtainable.

With regard to empirical and mechanistic studies, in addition to stereochemical structure and pK_a values it is desirable to ascertain the NMR chemical shifts of the aldehydic protons of the various PAM compounds. In Section II we specified a chemical

shift of 8.45 ppm (from DSS internal standard in D_2O solvent) for the aldehydic proton of 3-F-4-PAM. In the current quarter we have obtained the analogous chemical shift values for 2-PAM and 4-PAM: 8.69 ppm for 2-PAM and 8.37 ppm for 4-PAM. (See Figs. 9 and 10 for the pertinent NMR spectra.) These chemical shift values reflect relative deshielding and correlate with the relative acidities of the oximes. Compared with 4-PAM (aldehydic proton at 8.37 ppm) it is seen that the aldehydic proton of 3-F-4-PAM (8.45 ppm) is more deshielded, as expected from the electron-withdrawing effect of the F- atom. The aldehydic proton of 2-PAM is still more deshielded (8.69 ppm) owing to its closer position in the bonding framework to the positive nitrogen of the pyridine ring. For purposes of systematic study it would be desirable to additionally determine the chemical shift for TMB-4.

Further NMR studies carried out during this quarter comprised a brief investigation into the comparative H-bonding behavior of the dissociable (oxime) protons of 3-F-4-Pyridinecarboxaldehyde oxime, 4-Pyridinecarboxaldehyde oxime, and 2-Pyridinecarboxaldehyde oxime (hereafter referred to as 3-F-4-oxime, 4-oxime, and 2-oxime respectively). We found that these compounds (as opposed to their more polar quaternized (PAM) derivatives) are sufficiently soluble in nonpolar NMR solvent ($CDCl_3$) to obtain good NMR spectra therein and that these spectra show a well-defined peak corresponding to the oxime proton as well as one corresponding to the aldehydic proton. (Note that in D_2O solvent, as expected and previously reported, the oxime proton is washed out by deuterium exchange but the aldehydic proton is not.) We examined 0.04 Molar and 0.02 Molar solutions of each of the oximes in $CDCl_3$ solvent to determine whether there is a dilution effect. We do not wish in this Section to interpret in detail the spectra obtained (see Figs. 11 through 16), but we do wish to note our main conclusions. The results indicate that the dissociable proton of the 2-oxime

(Figs. 15 and 16, at 8.29 ppm from TMS) is not H-bonded since it is both relatively highly shielded and insensitive to dilution, whereas the corresponding proton of the 4-oxime is flagrantly H-bonded since it is both highly deshielded (9.56 ppm at 0.04 M) and highly sensitive to dilution (see Figs. 13 and 14). The analogous proton of 3-F-4-oxime (Figs. 11 and 12) also is clearly H-bonded, but apparently is a less advanced case. All of the H-bonding in this study is of the intermolecular type and dilution does not disrupt but apparently only causes the H distance to contract towards the oxime oxygen, causing greater shielding and thus an upfield shift of the intact peak. The fact that the proton of the 2-oxime is unaffected by dilution could be inter-preted as due to intramolecular H-bonding but its relatively high field position (compared to that of the 4-oxime and 3-F-oxime) implies the contrary, that it is not H-bonded at all. One expla-nation for an absence of H-bonding in this compound could be lack of accessibility of the pyridine nitrogen as an H-acceptor due to steric crowding from the 2- substituent. Another explanation is that the 2-oxime is simply less acidic than the other compounds, and its H insufficiently prototopic to bridge to an acceptor. In the case of 3-F-4-oxime, intramolecular H- bridging from the oxime oxygen to the F-atom is clearly not possible if the oxime has the syn-geometry.

A final observation in these spectra is that in comparing the aldehydic protons of these oximes, the chemical shift of that of the 3-F-4-oxime (8.35 ppm, Fig.s 11 and 12, unaffected by dilution) is greater than that of the 4-oxime (8.10 ppm, Figs. 13 and 14) as expected from the F-atom electron-withdrawing effect. However, the chemical shift of the 2-oxime aldehydic proton (7.77 ppm, Figs. 15 and 16) is strikingly less than that of these other compounds. This is consistent with the acidity of the 2-oxime being substantially less, perhaps sufficiently so to stop the oxime proton from engaging in H- bridging. Upon quaternization

to form 2-PAM it is evident that the acidity of this same oxime group surpasses the others owing to its adjacency to the positively charged nitrogen atom of the pyridine ring.

32

ADDENDUM

ELEMENTAL ANALYSIS REPORT (SCHWARZKOPF MICROANALYTICAL LABORATORY, WOODSIDE, NY 11377):

3-F-4-PYRIDINECARBOXALDEHYDE OXIME
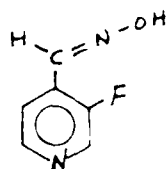
$C_6H_5N_2OF$

MW 140.12

	THEORY	FOUND
%C	51.43	51.54
%H	3.59	3.59
%N	20.00	20.28
%F	13.56	13.50

3-F-4-PYRIDINECARBOXALDEHYDE OXIME-METHIODIDE
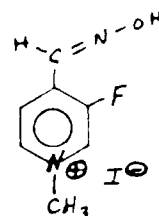
$C_7H_8N_2OFI$

MW 282.06

	THEORY	FOUND
%C	29.81	29.90
%H	2.86	3.10
%N	9.93	9.45
%F	6.74	7.03
%I	44.99	44.71

CFT-20 SPECTRUM NO. J-16
OPERATOR J.M.Lit DATE shifts

SAMPLE 2-PAM (chemical dept
Signa Co.)
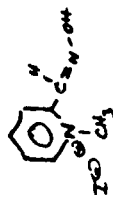
2-PAM

in D₂O solvent
(DSS int std)
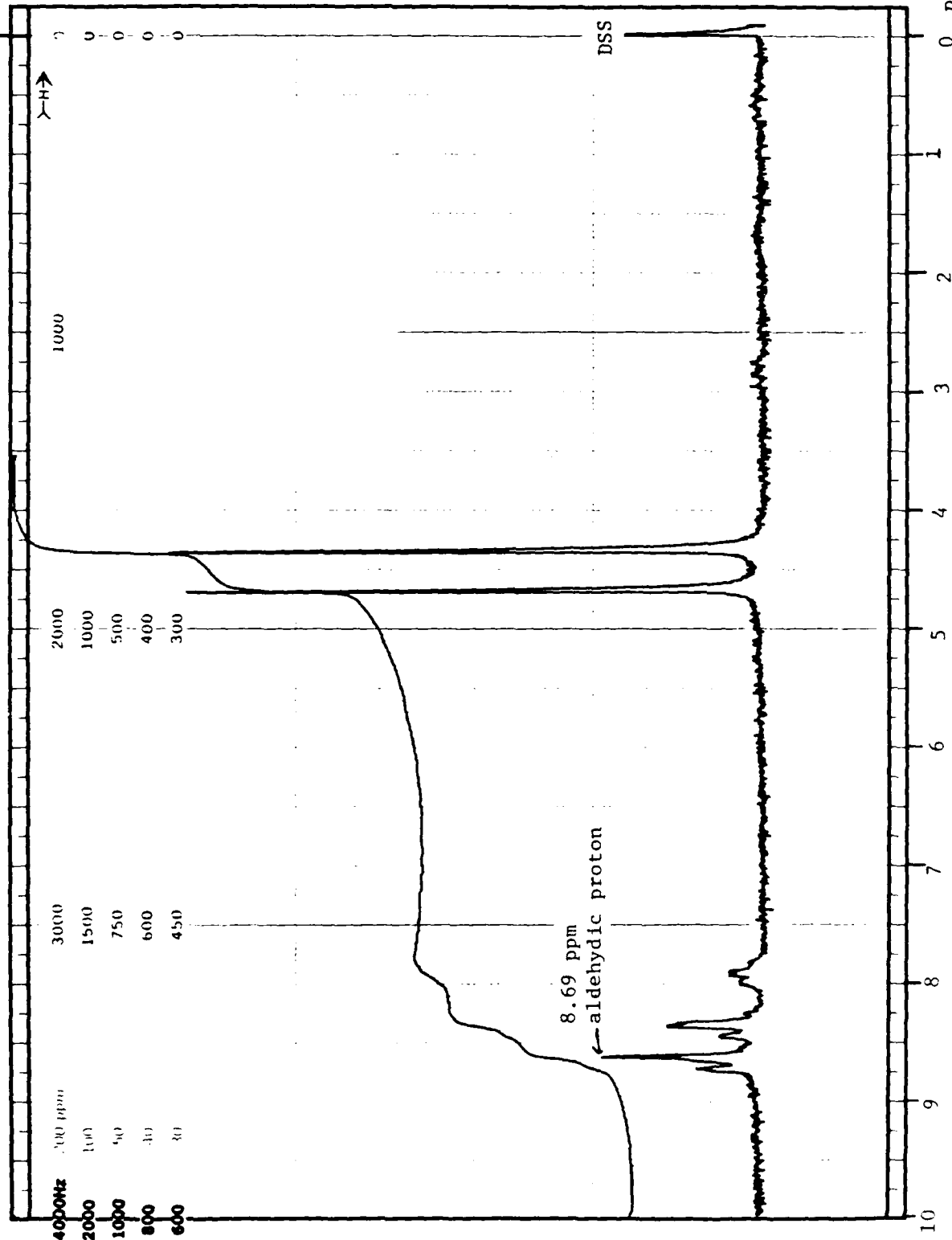
LOCK SIGNAL D₂O SPIN RATE 6 rps TEMP ___ °C

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO. OF TRANSIENTS (NT) 25
ACQUISITION TIME (AT) 2.0 sec
PULSE WIDTH (PW) 45 usec
PULSE DELAY (PD) 0 sec
DATA POINTS (DP) 4000

TRANSMITTER OFFSET (TO) 46
HIGH FIELD ✓ LOW FIELD X
RECEIVER GAIN (RG) X

DECOUPLER MODE (DM) ___
DECOUPLER OFFSET (DO) ___
NOISE BANDWIDTH (NB) ___ kHz

DISPLAY
SENS. ENHANCEMENT (SE) 0 sec
WIDTH OF PLOT (WP) 50 Hz
END OF PLOT (EP) -50 Hz
WIDTH OF CHART (WC) 500 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 100
REFERENCE LINE (RL) 0.00 (DSS)

varian
PRTD IN U.S.A.

33

DSS

1000

2000
1000
500
400
300

3000
1500
750
600
450

4000Hz
2000
1000
800
600

8.69 ppm
aldehydic proton

0 ppm
chemical
shift

Proton NMR spectrum of 2-PAM in deuterium oxide solvent
for chemical shift of aldehydic proton

Fig. 9

CFT-20 SPECTRUM NO. J-15

OPERATOR J McLick DATE 5/29/82

SAMPLE 4-PAM, prepared 4 JM
from 4-pyridinecarboxaldehyde
and (CH₃)₃ perchlorate
with methyl iodide
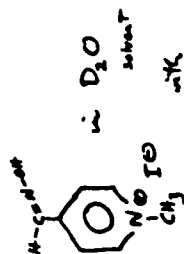
in D₂O
solvent

4-PAM

LOCK SIGNAL D₂O
SPIN RATE 6 rps.   TEMP. amb. °C

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO. OF TRANSIENTS (NT) 15
ACQUISITION TIME (AT) 3.0 sec.
PULSE WIDTH (PW) 45 sec.
PULSE DELAY (PD) 0 sec.
DATA POINTS (DP) 8200

TRANSMITTER OFFSET (TO) 46
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 5

DECOUPLER MODE (DM)
DECOUPLER OFFSET (DO)
NOISE BANDWIDTH (NB) kHz

DISPLAY
SENS. ENHANCEMENT (SE) 0 sec.
WIDTH OF PLOT (WP) 950 Hz
END OF PLOT (EP) -50 Hz
WIDTH OF CHART (WC) 900 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 100 Hz
REFERENCE LINE (RL) 0.00 (ppm)

varian
PRINTED IN U.S.A.

34

DSS

4.36 ppm
-CH₃ group
on pyridine
nitrogen

4.72 ppm
hydroxyl
impurity
in D₂O

8.37 ppm
aldehydic proton

Pyridine ring
protons 3 and 5

ring
protons
2 and 6

0 ppm
chemical
shift

Proton NMR spectrum of 4-PAM in deuterium oxide solvent
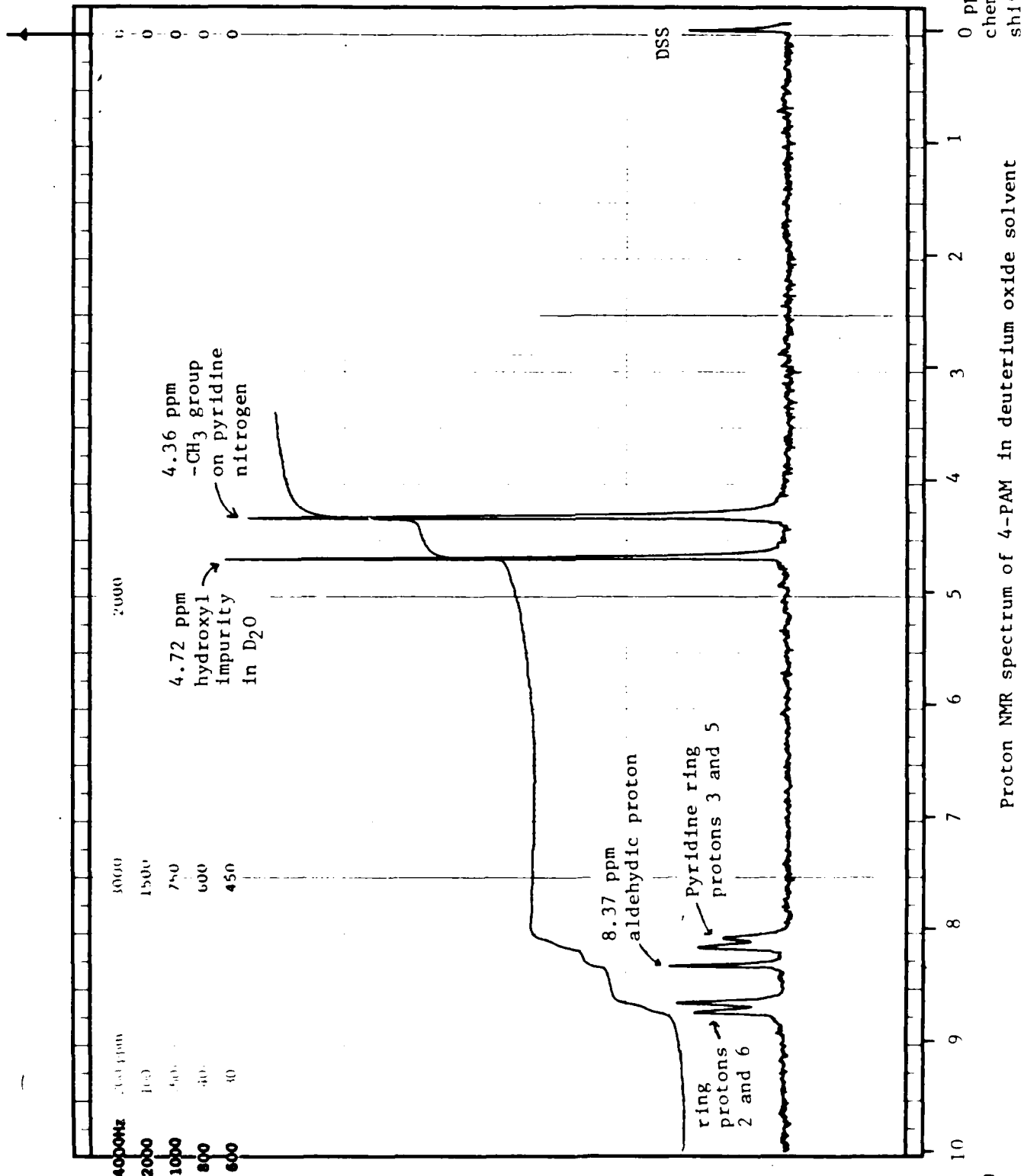
Fig. 10

CFT-20 SPECTRUM NO. *J-9*

OPERATOR *J.Malick* DATE *7.-1/7-*

SAMPLE *1*

3-F-4-Pyridinecarbaxaldehyde oxime

0.040 Molar sola,
~ CDCl₃ solvent
wih Tms ret. mtl.

LOCK SIGNAL _*CDCl₃*_
SPIN RATE _*6*_ rps. TEMP _*amb.*_ °C

ACQUISITION
SPECTRAL WIDTH (SW) _*1000*_ Hz
NO. OF TRANSIENTS (NT) _*25*_
ACQUISITION TIME (AT) _*2.025*_ sec.
PULSE WIDTH (PW) _*95*_ sec.
PULSE DELAY (PD) _*1.0*_ sec.
DATA POINTS (DP) _*free*_

TRANSMITTER OFFSET (TO) _*94*_
HIGH FIELD ___✓___ LOW FIELD _____
RECEIVER GAIN (RG) _*8*_

DECOUPLER MODE (DM) _____
DECOUPLER OFFSET (DO) _____
NOISE BANDWIDTH (NB) _____ kHz

DISPLAY
SENS. ENHANCEMENT (SE) _*-1.5*_ sec.
WIDTH OF PLOT (WP) _*950*_ Hz
END OF PLOT (EP) _*-50*_ Hz
WIDTH OF CHART (WC) _*800*_ Hz
END OF CHART (EC) _*0*_ Hz
VERTICAL SCALE (VS) _*50*_
REFERENCE LINE (RL) _*0.00 (Tms)*_

(V A) varian

0 ppm
chemical
shift

TMS

hydroxyl impurities
omitted

8.35 ppm
aldehydic proton

7.26 ppm
CHCl₃ impurity
in CDCl₃ solvent

8.96 ppm
oxime OH
proton

4000Hz   3000   2000
2000   1500   1000
1000   750   500
          600   400
          400   300

Proton NMR spectrum of 3-F-4-pyridinecarboxaldehyde oxime
(0.040 Molar solution in CDCl₃)

Fig. 11

Proton NMR spectrum of 3-F-4-pyridinecarboxaldehyde oxime
(0.020 Molar solution in CDCl$_3$)

Fig. 12

CFT-20 SPECTRUM NO. J-11

OPERATOR J. McLick DATE 9/24/77

SAMPLE 2

4-Pyr carboxaldehyde oxime

0.040 Molar soln.,

CDCl₃ solvent

TMS int. std.

LOCK SIGNAL CDCl₃

SPIN RATE 6 rps. TEMP ≈t °C

ACQUISITION

SPECTRAL WIDTH (SW) 1000 Hz

NO. OF TRANSIENTS (NT) 25

ACQUISITION TIME (AT) sec

PULSE WIDTH (PW) 45 μsec

PULSE DELAY (PD) 1.0 sec

DATA POINTS (DP) 8192

TRANSMITTER OFFSET (TO) 99

HIGH FIELD ___ LOW FIELD 0

RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) ___

DECOUPLER OFFSET (DO) ___

NOISE BANDWIDTH (NB) ___ kHz

DISPLAY

SENS. ENHANCEMENT (SE) -1.5 sec.

WIDTH OF PLOT (WP) 850 Hz

END OF PLOT (EP) -50 Hz

WIDTH OF CHART (WC) 900 Hz

END OF CHART (EC) 0 Hz
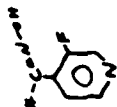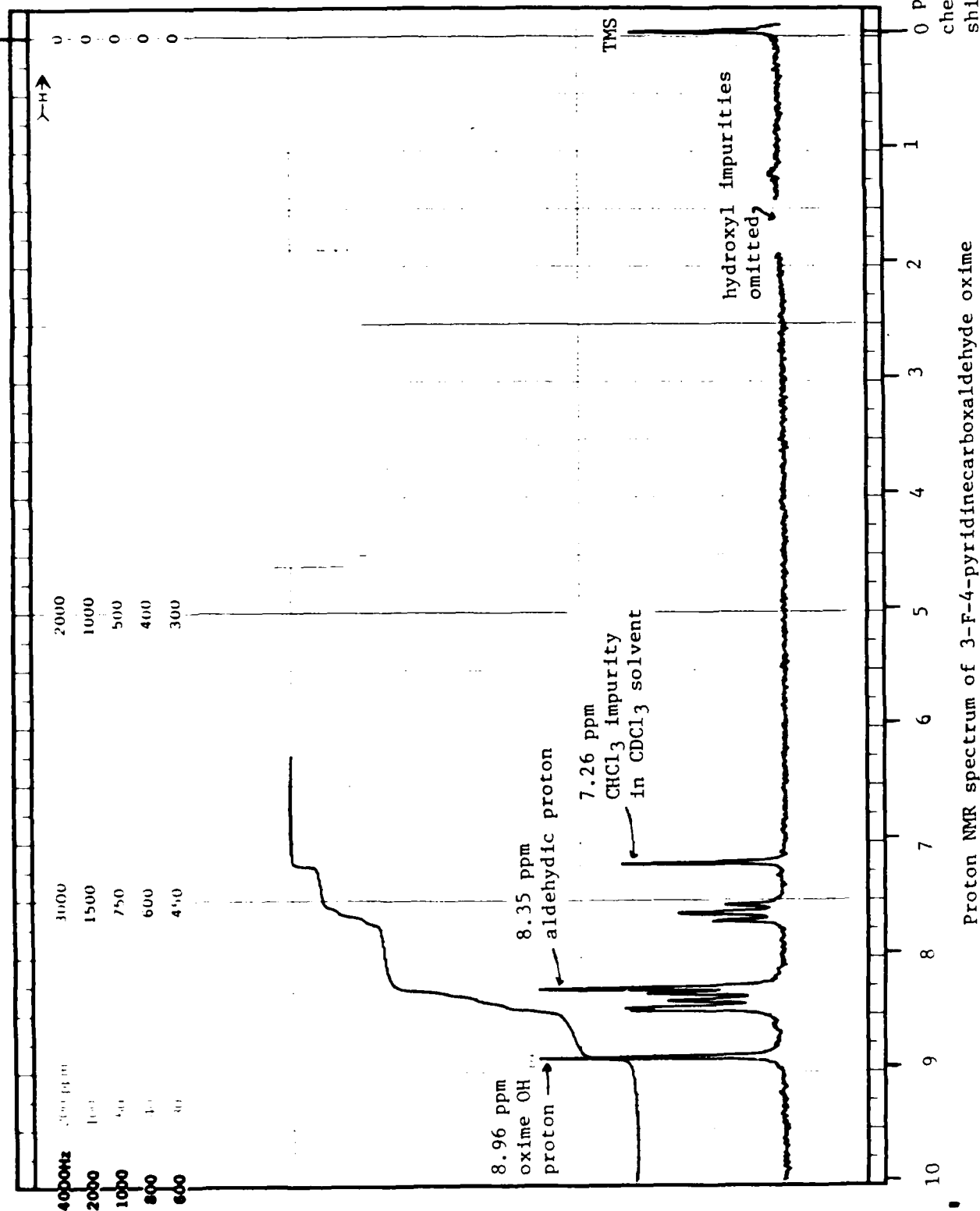
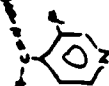VERTICAL SCALE (VS) 50

REFERENCE LINE (RL) 0.00 (TMS)

37

varian

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0 ppm

chemical shift

TMS

hydroxyl impurities omitted here

8.11 ppm aldehydic proton

7.26 ppm CHCl₃ impurity in CDCl₃ solvent

9.56 ppm oxime OH proton

4000Hz   2000   1000   800   600

Proton NMR spectrum of 4-pyridinecarboxaldehyde oxime
(0.040 Molar solution in CDCl₃)

Fig. 13

CFT-20 SPEC   1 NO. J-12
OPERATOR   DATE 4/24/8?

SAMPLE 2 2
Acenon

4-Pyrcarboxaldehyde oxime

0.020 Molar soli

CDCl₃ used
TMS int. std.

LOCK SIGNAL CDCl₃
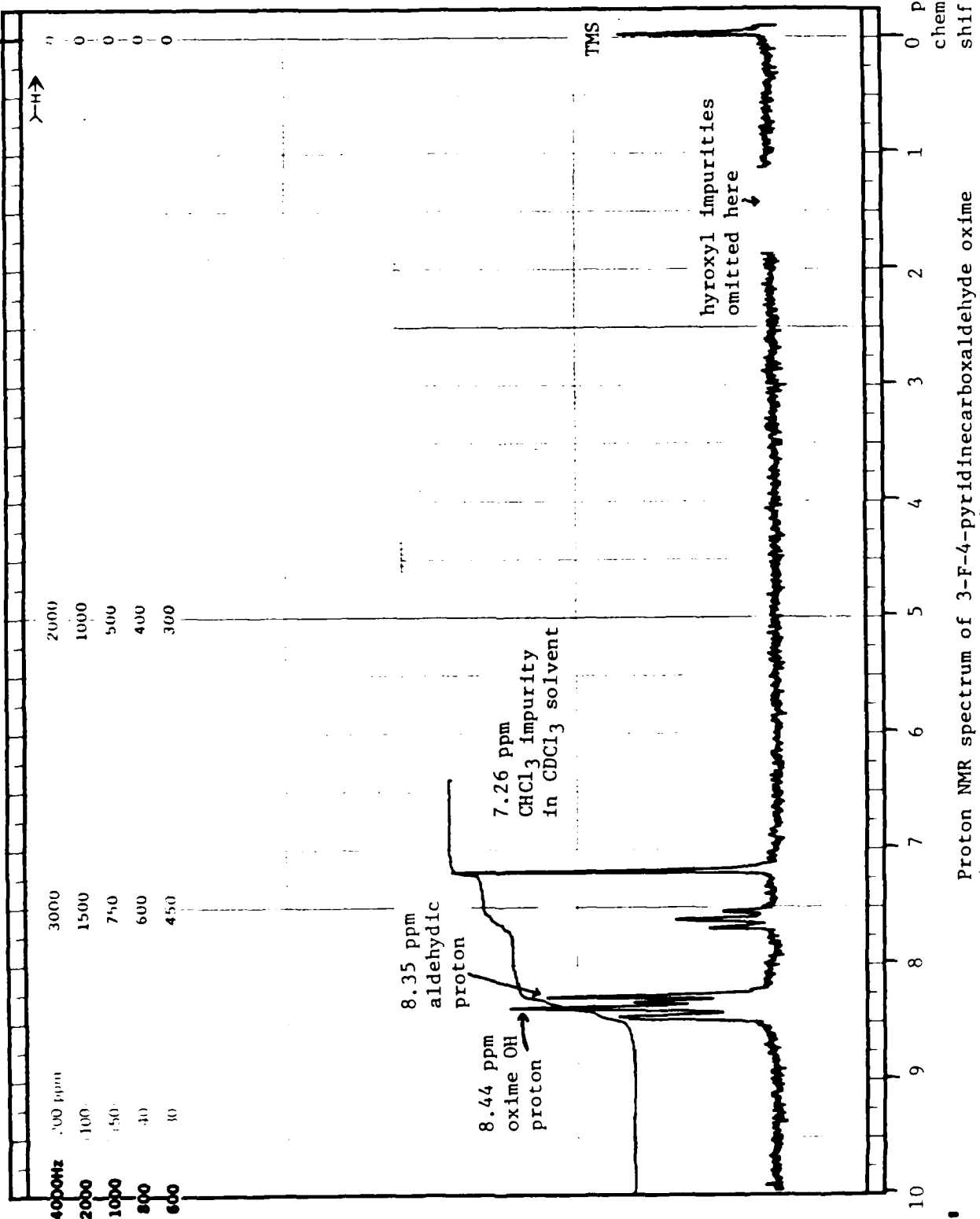SPIN RATE 6 rps.   TEMP 25±2°C

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO. OF TRANSIENTS (NT) 25
ACQUISITION TIME (AT) 4.095 sec.
PULSE WIDTH (PW) 42 sec.
PULSE DELAY (PD) 1.0 sec.
DATA POINTS (DP) 8192

TRANSMITTER OFFSET (TO) 47
HIGH FIELD ✓   LOW FIELD
RECEIVER GAIN (RG) 2

DECOUPLER MODE (DM)
DECOUPLER OFFSET (DO)
NOISE BANDWIDTH (NB) kHz

DISPLAY
SENS. ENHANCEMENT (SE) 7.5 sec.
WIDTH OF PLOT (WP) 850 Hz
END OF PLOT (EP) -50 Hz
WIDTH OF CHART (WC) 100 Hz
END OF CHART (EC) 50 Hz
VERTICAL SCALE (VS) 100
REFERENCE LINE (RL) 0.00 (TMS)

varian
PRINTED IN U.S.A.

38
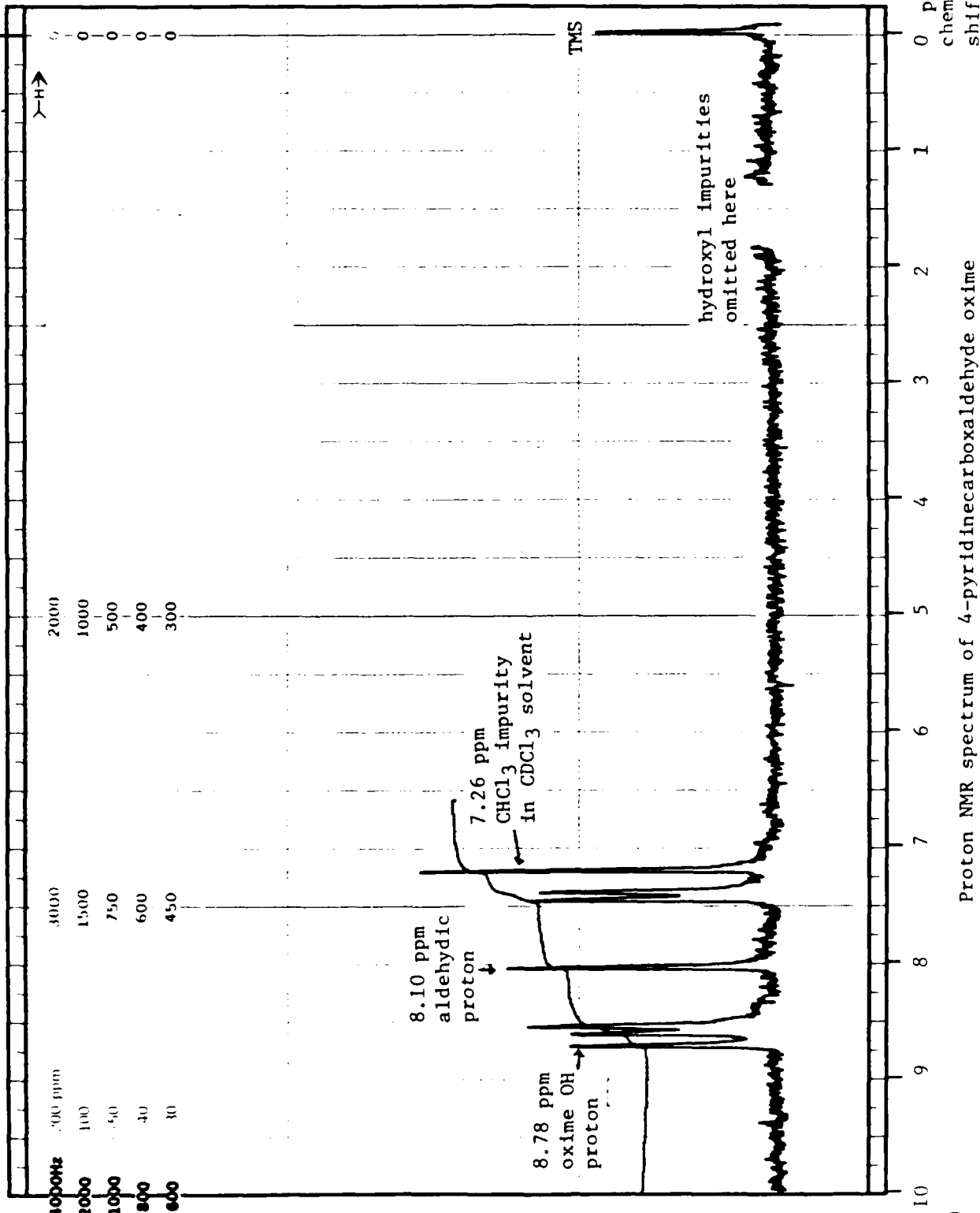
Proton NMR spectrum of 4-pyridinecarboxaldehyde oxime
(0.020 Molar solution in CDCl₃)

Fig. 14

CFT-20 SPECTf   NO. J-13
OPERATOR   DATE 9/07/82
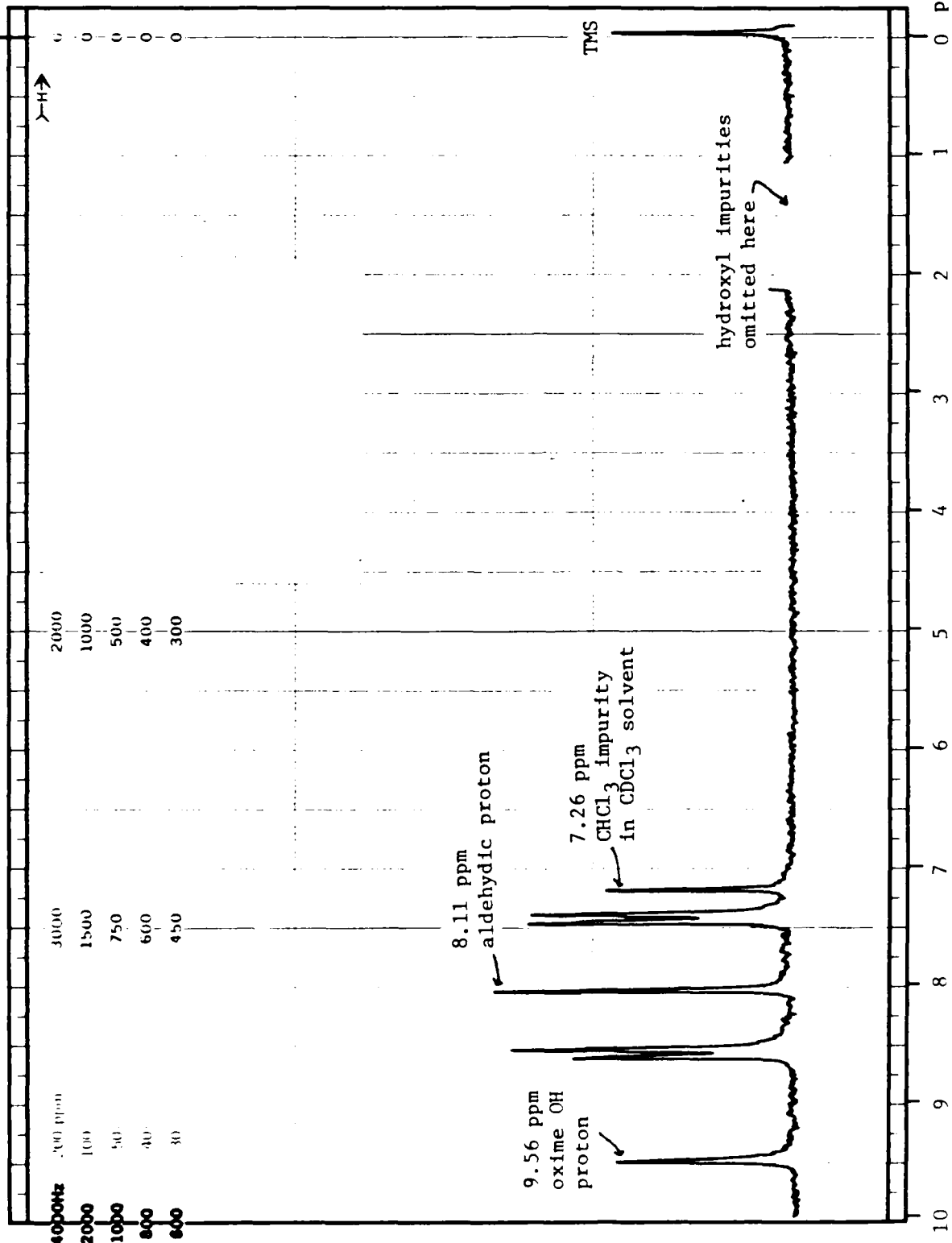
SAMPLE 3

2-Pyr carboxaldehyde oxime
0.040 Molar soln;

CDCl₃ solvent
TMS int. std.

LOCK SIGNAL   CDCl₂
SPIN RATE 6. rps.   TEMP amb. °C

ACQUISITION
SPECTRAL WIDTH (SW) ____ 1000 ___ Hz
NO OF TRANSIENTS (NT) ___ 25 ___
ACQUISITION TIME (AT) ___ 4.05 ___ sec.
PULSE WIDTH (PW) ___ 45 ___ sec.
PULSE DELAY (PD) ___ 1.0 ___ sec.
DATA POINTS (DP) ___ 8100 ___

TRANSMITTER OFFSET (TO) 44
HIGH FIELD ✓   LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) ____
DECOUPLER OFFSET (DO) ____
NOISE BANDWIDTH (NB) ____ kHz

DISPLAY
SENS ENHANCEMENT (SE) ___ -1.5 ___ sec.
WIDTH OF PLOT (WP) ___ 850 ___ Hz
END OF PLOT (EP) ___ -50 ___ Hz
WIDTH OF CHART (WC) ___ 600 ___ Hz
END OF CHART (EC) ___ 50 ___ Hz
VERTICAL SCALE (VS) ___ 50 ___
REFERENCE LINE (RL) ___ 0.00 (7~1) ___

0 ppm
chemical shift

varian
printed in U.S.A.

TMS

hydroxyl impurity

7.26 ppm
CHCl₃ impurity
in CDCl₃ solvent

7.77
ppm

8.29
ppm

Proton NMR spectrum of 2-pyridinecarboxaldehyde oxime
(0.040 Molar solution in CDCl₃)

Fig. 15

4000Hz   2000
2000   1000
1000   500
800   400
600   300

CFT-20 SPECTRL NO. J-14

OPERATOR ___ DATE _____

SAMPLE 3~

2-Pyridinecarboxaldehyde oxime

0.020 M sol.

CDCl₃ solvent
TMS int. std.

ACQUISITION
SPECTRAL WIDTH (SW) ___ 1000 ___ Hz
NO. OF TRANSIENTS (NT) ___ 25
ACQUISITION TIME (AT) ___ sec.
PULSE WIDTH (PW) ___ sec.
PULSE DELAY (PD) ___ 1.0 ___ sec.
DATA POINTS (DP) ___ 8100

LOCK SIGNAL _CDCl₃_
SPIN RATE _8_ rps

TRANSMITTER OFFSET (TO) _49
HIGH FIELD ___ LOW FIELD _P_
RECEIVER GAIN (RG) ___ P

DECOUPLER MODE (DM)
DECOUPLER OFFSET (DO)
NOISE BANDWIDTH (NB) ___ kHz

DISPLAY
SENS. ENHANCEMENT (SE) _-1.5_ sec.
WIDTH OF PLOT (WP) _150_ Hz
END OF PLOT (EP) _-50_ Hz
WIDTH OF CHART (WC) _P_ Hz
END OF CHART (EC) _50_ Hz
VERTICAL SCALE (VS) _100_
REFERENCE LINE (RL) _0.00 (TMS)_

Varian
MADE IN U.S.A.

40

TMS

hydroxyl impurities

7.25 ppm
CHCl₃ impurity
in CDCl₃ solvent

7.77 ppm

8.26 ppm

0 ppm chemical shift

Proton NMR spectrum of 2-pyridinecarboxaldehyde oxime
(0.020 Molar solution in CDCl₃)

Fig. 16

4000Hz
2000
1000
800
600

2000
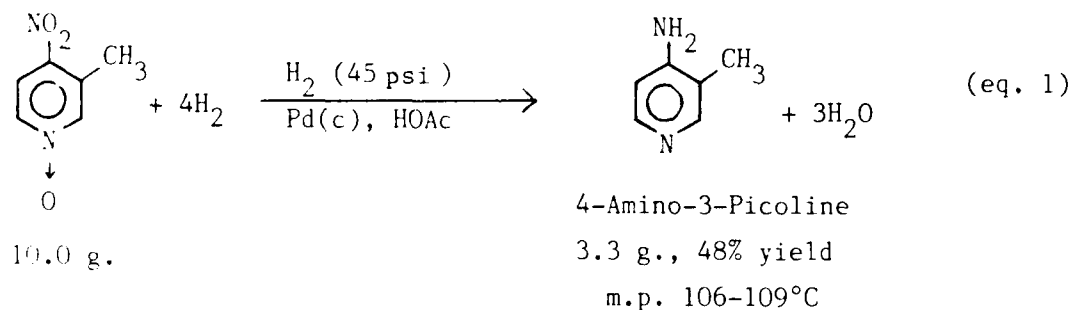1500
750
600
450

3000
1000
500
400
300

Section IV

Fourth Quarter: June 1 – August 31, 1982

During this quarter chemical synthesis experiments were continued, spectroscopic studies of PAM compounds were extended, and chemical reactivity tests were initiated.

In Section III, Third Quarter, the first synthetic step towards 3-F-2-PAM and the first synthetic step towards 4-F-3-PAM were each carried out. Regarding 3-F-2-PAM, as explained previously, the second step could not be embarked on until we obtained a steam generator of suitable capacity requisite to steam-distill nitration product isomers A and B. We ordered an appropriate steam generator from VWR Scientific, Inc., but due to a delay by the manufacturer the unit has not yet been received, and therefore during this quarter no further work towards 3-F-2-PAM was carried out.

For 4-F-3-PAM, its second synthetic step, reduction of 4-Nitro-3-Picoline-N-oxide to 4-Amino-3-Picoline, was achieved in the present quarter by catalytic hydrogenation in the Parr aparatus:

$$\text{(NO}_2\text{-3-CH}_3\text{-pyridine-N-oxide)} + 4H_2 \xrightarrow[\text{Pd(c), HOAc}]{H_2 \text{ (45 psi)}} \text{(NH}_2\text{-3-CH}_3\text{-pyridine)} + 3H_2O \quad \text{(eq. 1)}$$

10.0 g.

4-Amino-3-Picoline

3.3 g., 48% yield

m.p. 106–109°C

The next synthetic step is conversion of 4-Amino-3-Picoline to 4-Fluoro-3-Picoline by way of the Schiemann reaction:

$$\xrightarrow[\text{aq. HBF}_4, \text{ cold}]{\text{Diazotization}} \xrightarrow[\text{decomp.}]{\text{warm}} + N_2\uparrow + BF_3$$

Diazonium

Borofluoride

Salt

4-F-3-Picoline

(eq. 2)
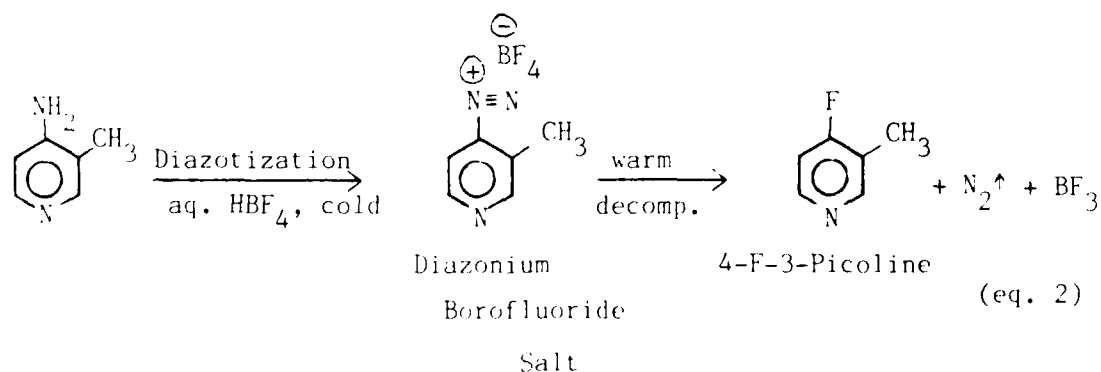
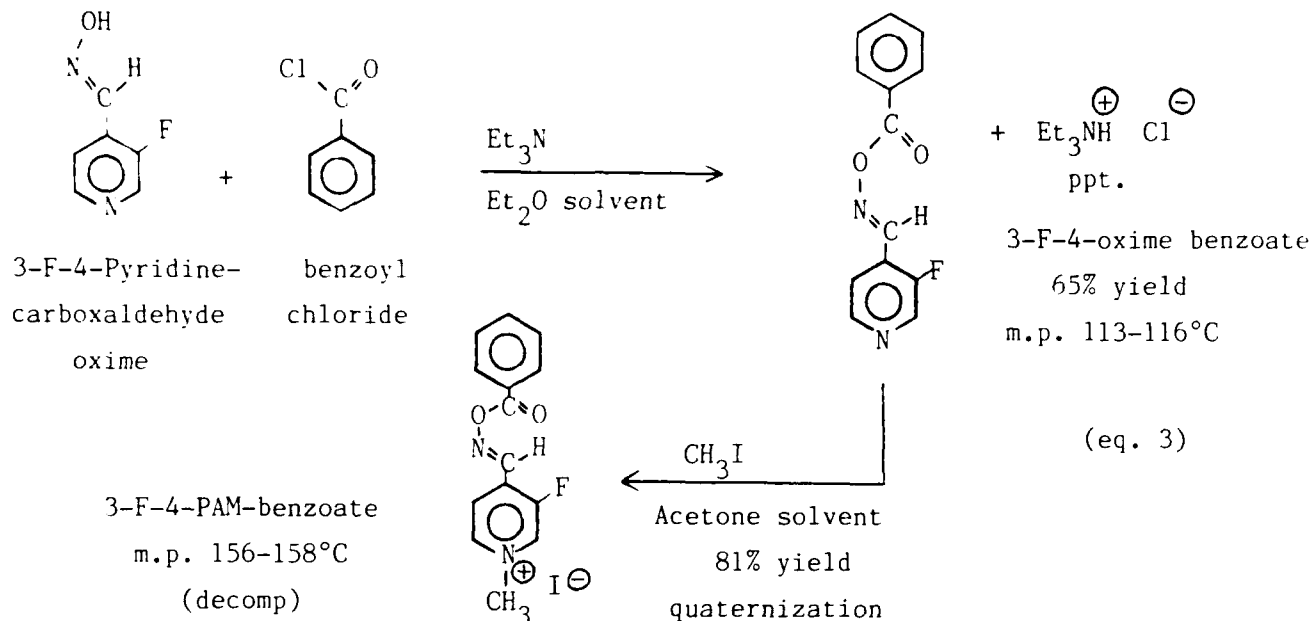We initially envisaged product 4-F-3-Picoline based on a citation in the literature of the previous synthesis of that F-Picoline.[14] However, these authors do not give fundamental data for this compound. Using $NaNO_2$ as the diazotization agent, the diazonium borofluoride salt (see eq. 2 above) did not precipitate and as a result it was not isolated and dried. Instead Rowbotham and Schaefer carried out the decomposition step _in situ_ by warming the initial diazotization solution (aqueous). Upon work-up the authors obtained a "poor" yield (unquantified, presumably less than 10%) of the purported 4-F-3-Picoline. Such a poor yield is understandable because the presence of water, even in the form of moisture, during decomposition lowers the yield considerably. It is authoritatively known that diazonium borofluoride salts must be thoroughly dried before decomposition (see Ref. 16).

In an effort to improve this step to an acceptable yield we carried out the diazotization using EtONO as the diazotization agent. At 0 - 5°C no precipitation occurred, but on chilling the mixture to -70°C substantial white precipitate developed. This material was isolated, dried, and subjected to decomposition (warming) conditions under hydrocarbon (n-hexane), whereupon evidence of N_2 evolution was observed (generation of bubbles well below the bp of n-hexane). However, final work-up gave only 1% of the theoretical weight of a material which has not yet been rigorously identified. It should be noted that Roe and Hawkins[17] in their systematic studies on the Schiemann reaction were unsuccessful in their attempts to find a procedure to reliably isolate 4-Pyridine diazonium borofluoride, nor were they ever able to isolate 4-F-Pyridine (see Roe and Hawkins, _op_. _cit_.). Additionally in their systematic studies, which included F-Picolines, they never reported 4-F-3-Picoline. On the unsubstituted pyridine ring it is known that F in the 4- position is unstable (Roe and Hawkins, _op_. _cit_.), and our results confirm this for 4-F in methyl-substituted pyridines. Unless in our laboratory a method is developed to obtain 4-F-3-Picoline, synthesis of 4-F-3-PAM in pragmatically useful amounts will not be practical. We are investigating into this situation further.

Additional synthesis work in this quarter comprised derivatization of 3-F-4-PAM and 4-PAM as their respective benzoates. This was achieved by first benzoylating the unquaternized oximes and then quaternizing the benzoates. as shown in eq. 3 below, starting with 3-F-4-Pyridinecarboxaldehyde oxime.

OH

N=C–H

F

3-F-4-Pyridine-
carboxaldehyde
oxime

+

Cl–C=O

benzoyl
chloride

Et_3N

Et_2O solvent

→

C=O
O
N=C–H
F

+ $Et_3NH^{\oplus}$ $Cl^{\ominus}$
ppt.

3-F-4-oxime benzoate
65% yield
m.p. 113–116°C

(eq. 3)

O–C=O
N=C–H
F
N$^{\oplus}$ $I^{\ominus}$
CH_3

3-F-4-PAM-benzoate
m.p. 156–158°C
(decomp)

CH_3I

Acetone solvent
81% yield
quaternization

The resultant 3-F-4-PAM-benzoate is easily recrystallized from H_2O, and its
identity was verified by satisfactory elemental analysis (sample analyzed
by Schwarzkopf Microanalytical Laboratory, see Addendum in this section,
p. 48, for data).

The analogous 4-PAM-benzoate (mp 154 – 156°C) was synthesized for pur-
poses of comparison. This benzoate is cited by Hagedorn, Stark ad Lorenz
(Agnew. Chem. Internat. Ed. 11, 307-9 [1972]) but without any support data.
Therefore we obtained a proton NMR spectrum for this compound as well as
for 3-F-4-PAM-benzoate (see Figs. 17 and 18). Based on comparisons with the
NMR spectra of the corresponding unbenzoylated PAM compounds (see previous
Sections), we assign the resonance peak at 9.16 ppm in the spectrum of
3-F-4-PAM-benzoate to the aldehydic proton (see Fig. 17), and the resonance
peak at 9.01 ppm in the spectrum of 4-PAM-benzoate to its aldehydic proton
(see Fig. 18). It is observed that benzoylation has the effect of signif-
icantly shifting the aldehydic protons downfield, ie. benzoylation of
3-F-4-PAM shifts the proton from 8.45 ppm to 9.16 ppm, and benzoylation of
4-PAM shifts the proton from 8.37 ppm to 9.01 ppm. Additionally, as in the
unbenzoylated compounds, the aldehydic proton of the F-compound continues to
resonate downfield from the non-F-compound, ie. the F atom continues to
deshield the aldehydic proton.

Structural studies relating to 3-F-4-PAM were strongly augmented in
this quarter by X-ray crystal analysis of 3-F-4-Pyridinecarboxaldehyde oxime,
the unquaternized parent compound of 3-F-4-PAM. This analysis was done in

collaboration with Dr. Jenny P. Glusker and her group at the Institute for Cancer Research, Philadelphia. The geometry of the oxime is <u>syn</u>, which is the geometry commonly found to prevail in the pyridinecarboxaldehyde oximes. Furthermore the aldehydic H atom is located on the same side of the molecule as the F atom on the pyridine ring.
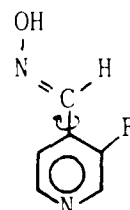

(eq. 4)

The torsional angle between the planar oxime group and the planar pyridine ring is near $0°$, ie. the two groups are essentially co-planar. A diagram which depicts the molecules in the crystal lattice is included in this report (Fig. 19).

As desirable preliminary data for chemical reactivity studies and because such knowledge may be useful in other regards, we obtained the UV absorption spectra of 3-F-4-PAM, 4-PAM, and 2-PAM. Using our Perkin-Elmer 552 UV-Vis spectrophotometer attached to a Perkin-Elmer/Hitachi 057 X-Y recorder, the spectra were obtained from 0.04 mMolar solutions of the PAM compounds in 20 mM sodium phosphate buffer, pH 7.40, contained in 1.0 cm pathlength cuvettes. The absorption of the three compounds, displayed simultaneously for comparison, are given in Fig. 20. Each compound shows 4 absorption bands, and these are analogous from compound to compound. We refer to these as Bands 1, 2, 3, and 4, which are numbered progressively with decreasing wavelength (see Fig. 20). For 3-F-4-PAM the Υ_{max} and molar extinction coefficient (ϵ) for each band are as follows: Band 1 (348nm, ϵ 3,750), Band 2 (286nm, ϵ 11,900), Band 3 (226nm, ϵ 15,200) and Band 4 (199nm, ϵ 16,500). The Υ_{max} values for the other compounds are indicated in Fig. 20 and their ϵ values are of the same orders of magnitude as those listed above for 3-F-4-PAM. Such complete spectra of 2-PAM and 4-PAM have not been reported by Hagedorn's laboratory, and our study may comprise the first comparative study.

We assign Band 1 to what Hagedorn, Gundel and Schoene[18] refer to as the "Betaine" chromophore, ie. the conjugate base from which the acidic proton has dissociated. This is pictured below for 3-F-4-PAM, including resonance stabilization:
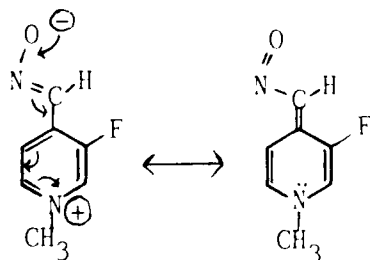
(eq. 5)

We assign Band 2 to undissociated pyridinium oxime (ie. with the proton still bonded to the oxime oxygen atom) and we assign Bands 3 and 4 to the aromatic pyridinium chromophore. These latter two bands are not dependent on the presence of the oxime group, although their exact absorption maxima (wavelength and ϵ) are subject to perturbations by oxime or other ring substituents.

In comparing the spectra of the three PAM compounds in Fig. 20 it is noteworthy that with respect to Bands 1 and 2 3-F-4-PAM resembles 2-PAM more closely than 4-PAM. Similar populations of dissociated and undissociated species for 3-F-4-PAM and 2-PAM are expected on the basis of their pK_a values, whereas the corresponding population of dissociated 4-PAM is considerably smaller owing to a higher pK_a. Therefore the intensity of Band 1 for 4-PAM is markedly less than for 3-F-4-PAM and 2-PAM, whereas the intensity of Band 2 for 4-PAM is correspondingly greater than for these other compounds.

By contrast, with respect to Bands 3 and 4, 3-F-4-PAM and 4-PAM show almost superimposable behavior. F-substitution has little effect on the aromatic pyridinium chromophore. The corresponding bands for 2-PAM are also quite similar, except for hyperchromicity in Band 3. Evidently Band 3 is sensitive to position of ring substitution.

Chemical reactivity studies initiated during this quarter fall in the two categories proposed for PAM compounds by Hagedorn and co-workers (Hagedorn, Gundel and Schoene (Ref. 18) and Hagedorn, Stark and Lorenz (Ref. 7). These are relatively simple, nonenzymatic model reactions which can be carried out in UV cuvettes and are purported to be useful in pre-testing the potential of new compounds as reactivators of poisoned acetylcholinesterase. The first of these model reactions is the reaction of PAM compounds with triethyl phosphate. Exact experimental details for this test have not been published in the available literature by Hagedorn et al., except that it is carried out in an aqueous buffer solution at pH 7.4 - 7.8 with an "excess" of triethyl phosphate, and followed in the UV by decay of what we refer to as Band 1 and 2 absorption. For TMB-4 the reaction is said to occur "in the course of several hours" (Hagedorn, Gundel and Schoene, op. cit.).

For our experiments with 2-PAM, 4-PAM, and 3-F-4-PAM, we employed 0.04 mMolar solutions of the PAM compounds in 20 mMolar sodium phosphate buffer at pH 7.40. Such solutions are suitable for observation of the UV spectra using common quartz cuvettes (1.0 cm pathlength). Preliminary experiments showed that 10-fold and 100-fold excesses of triethyl phosphate (pH held

constant at 7.40) had no effect on the UV spectra over periods of hours. At the cut-off of experimental work for this quarter we determined that even with a 1000-fold excess of triethyl phosphate there is little or no reaction with 2-PAM, 4-PAM, or 3-F-4-PAM even after 18 hours. Repetitive UV spectral scans for each of the PAM compounds exposed to 1000-fold triethyl phosphate are shown in Figs. 21, 22, and 23. In no case is there any substantive decay in Band 1 and 2 absorption. Therefore we conclude that Hagedorn's reactivity test for PAM compounds with triethyl phosphate does not work. In the Annual and Final Report, November 1983, First Quarter, we intend to investigate this purported reaction further, because if modifying conditions (eg. higher pH, metal ion catalysis) can be found to accelerate the reaction, it may still be a simple and useful test for reactivator potential.

The second model reaction proposed by Hagedorn and co-workers is the Beckmann decompostion reactivity test on PAM-benzoates (Hagedorn, Stark and Lorenz, Ref. 7). No experimental details have been published by these workers except that it is carried out in "aqueous solution at pH 8.1" and "determined by UV spectroscopy (Υ_{max} = 280nm)." We have measured the UV spectra of 3-F-4-PAM-benzoate and 4-PAM-benzoate in slightly acidic (pH 5.9) doubly-distilled H_2O in which the compounds are relatively stable (see Fig. 24). The Υ_{max} value for 3-F-4-PAM-benzoate is 270nm and that for 4-PAM-benzoate is 267nm. We followed the decompositions at these respective wavelengths (see Figs. 25 and 26) since they are relatively close to that used by Hagedorn et al. (280nm). At a pH buffered at 8.05 we found a half-life of 12 minutes for the UV absorption decay of 3-F-4-PAM-benzoate and a half-life of 60 minutes for that of 4-PAM-benzoate. This latter half-life for 4-PAM-benzoate (60 minutes) is considerably shorter than that reported for the same compound by Hagedorn, Stark and Lorenz (Ref. 7), 3600 minutes. To explain this discripancy we suggest that the German workers did not buffer their "aqueous solution" at pH 8.1. A proton is the co-product of each Beckmann decomposition event, and at a starting concentration of the 4-PAM-benzoate at 10^{-5} M this would be capable of neutralizing HO^- (10^{-6} M) after one-tenth of the Beckmann events, thus inhibiting base-catalysis of further decomposition.

We believe that the Beckmann decomposition test can be useful in comparing the chemical behavior of various PAM structures, but exact experimental conditions need to be specified by authors.

The intramolecular mechanism of the Beckmann decomposition is believed to

proceed as pictured below for <u>syn</u> 3-F-4-PAM-benzoate:

(eq. 6)
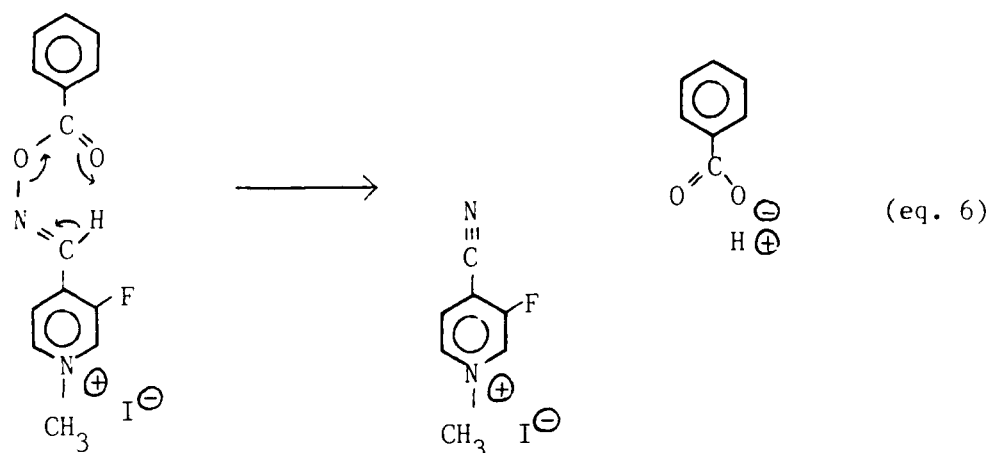
That esterified (including phosphonylated) PAM's readily undergo such an elimination cf their aldehydic H's (as depicted in eq. 6 above) whereas their unesterified counterparts (free oximes) do so only more slowly is consistent with the significant downfield shift (deshielding) those H's experience in their NMR spectra when the oxime group is benzoylated (as obser ed earlier in this report). Such deshielding renders the aldehydic H's more proton-like and thus more dissociable from their carbon atoms. Finally our prediction that this elimination would be accelerated by the presence of the F atom in the 3-position is confirmed by the present results.

<u>ADDENDUM</u>

ELEMENTAL ANALYSIS REPORT (SCHWARZKOPF MICROANALYTICAL LABORATORY, WOODSIDE, NY 11377):
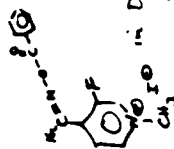
3-F-4-PAM-benzoate
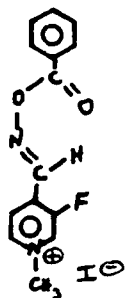
$C_{14}H_{12}N_2O_2FI$

MW 386.17

	THEORY	FOUND
% C	43.54	43.36
% H	3.13	3.31
% N	7.26	6.99
% F	4.92	5.21
% I	32.86	32.78

CFT-20 SPECTRUM NO. J-21
OPERATOR J. McLick DATE 6/9/92

SAMPLE 3-F-4-PAM-benzoate

H₂O in D₂O solvent,
w/x DSS
int. std.

NT: 200

LOCK SIGNAL D₂O
SPIN RATE 6 rps.   TEMPd=6 °C

ACQUISITION
SPECTRAL WIDTH (SW) .... 1000 .... Hz
NO. OF TRANSIENTS (NT) 200
ACQUISITION TIME (AT) ..... 4.095 .. sec
PULSE WIDTH (PW) .... 45 ..... .sec
PULSE DELAY (PD) .... 0 ...... sec
DATA POINTS (DP) 6000

TRANSMITTER OFFSET (TO) ... %
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) .... P

DECOUPLER MODE (DM)
DECOUPLER OFFSET (DO)
NOISE BANDWIDTH (NB) .... kHz

DISPLAY
SENS. ENHANCEMENT (SE) .. -2.5 . sec
WIDTH OF PLOT (WP) .... 850 .. Hz
END OF PLOT (EP) .... -50 .. Hz
WIDTH OF CHART (WC) ...... 900 .. Hz
END OF CHART (EC) .... 0 .... Hz
VERTICAL SCALE (VS) 250
REFERENCE LINE (RL) ...... 0.00 (DSS)

varian
PRINTED IN U.S.A.

4.49 ppm
-CH₃ group
on pyridine
nitrogen

4.69 ppm
OH impurity
in D₂O
solvent

9.16 ppm
aldehydic
proton

syn

DSS

0   1   2   3   4   5   6   7   8   9   10 ppm
chemical
shift

Proton NMR spectrum of 3-F-4-PAM-benzoate in deuterium oxide solvent

Fig. 17

4000Hz
2000
1000
800
600

CFT-20 SPECTRL NO. J-22

OPERATOR J. McLick DATE 8/8/82

SAMPLE 4-PAM-benzoate
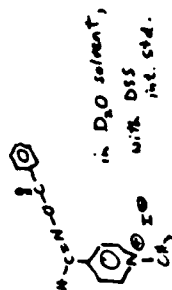
in D₂O solvent,
with DSS
int. std.

NT = 200

LOCK SIGNAL D₂O

SPIN RATE 6 rps.   TEMP.=6 °C

ACQUISITION

SPECTRAL WIDTH (SW) ___1000___ Hz

NO. OF TRANSIENTS (NT) ___200___

ACQUISITION TIME (AT) ___4.095___ sec.

PULSE WIDTH (PW) ___45___ μsec.

PULSE DELAY (PD) ___0___ sec.

DATA POINTS (DP) ___6000___

TRANSMITTER OFFSET (TO) 46

HIGH FIELD ✓   LOW FIELD

RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) _____

DECOUPLER OFFSET (DO) _____

NOISE BANDWIDTH (NB) _____ kHz

DISPLAY

SENS. ENHANCEMENT (SE) -2.5 sec.

WIDTH OF PLOT (WP) ___950___ Hz

END OF PLOT (EP) ___-50___ Hz

WIDTH OF CHART (WC) ___900___ Hz

END OF CHART (EC) ___0___ Hz

VERTICAL SCALE (VS) ___250___

REFERENCE LINE (RL) ___0.00___ (ppm)

varian

DSS

4.44 ppm
-CH₃ group
on pyridine
nitrogen

4.69 ppm
OH impurity
in D₂O
solvent

9.01 ppm
aldehydic
proton

0 ppm
chemical
shift

Fig. 18

Proton NMR spectrum of 4-PAM-benzoate in deuterium oxide solvent

Depiction of
3-F-4-pyridine-
carboxaldehyde
oxime in the
crystal lattice

Fig. 19

Comparison of the UV absorption spectra of 2-PAM, 4-PAM, and 3-F-4-PAM. (0.040 mM solutions in 20 mM phosphate buffer, pH 7.40). Each compound displays four absorption bands.
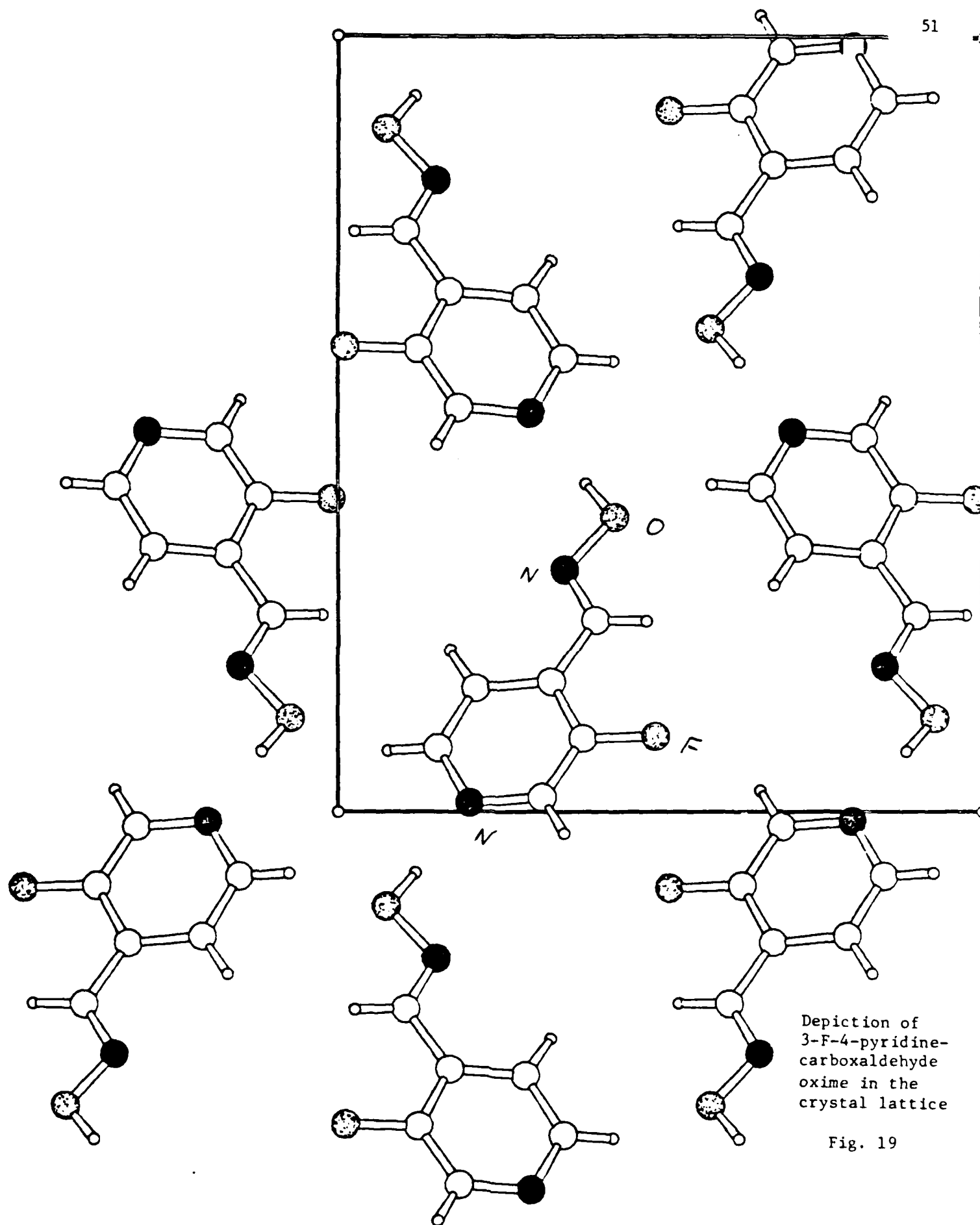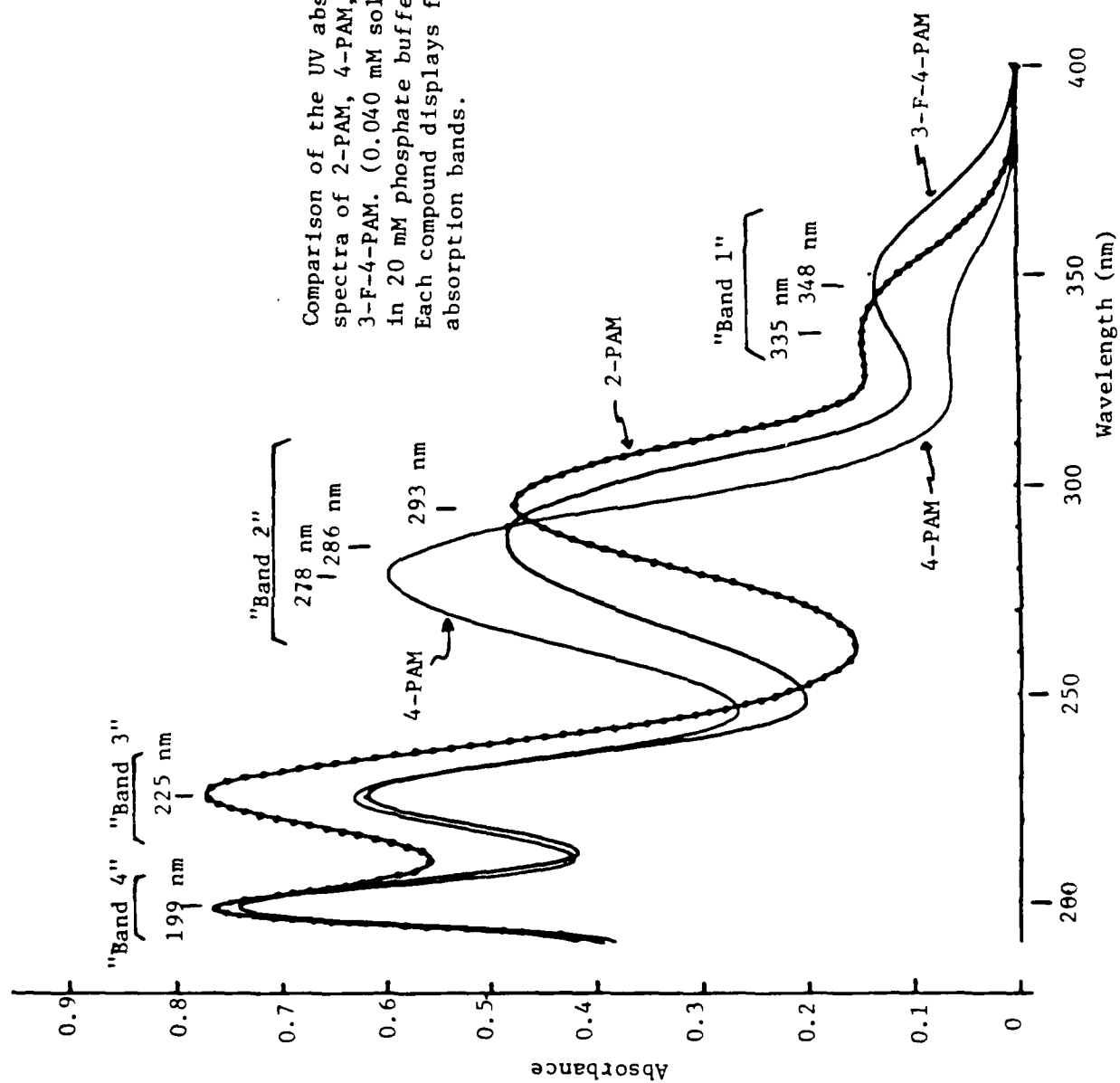
Fig. 20

53

Fig. 21

Exposure of 2-PAM to triethyl phosphate monitored by UV absorption spectra. UV absorption spectra of 2-PAM (i. 20 mM sodium phosphate buffer (pH 7.40) with a 1000-fold excess of triethyl phosphate). Repetitive scans of the UV spectrum. Final pH 7.40.
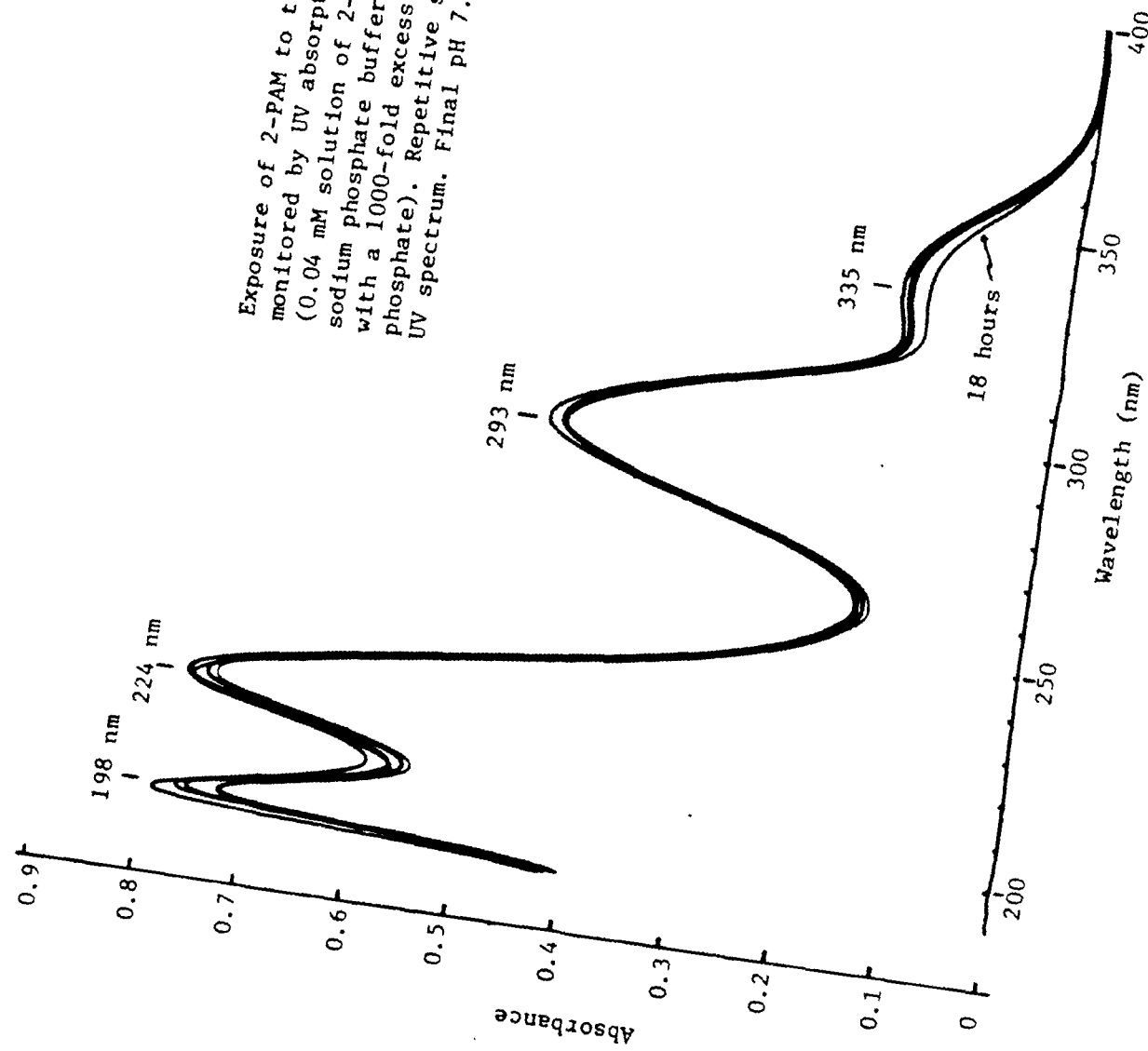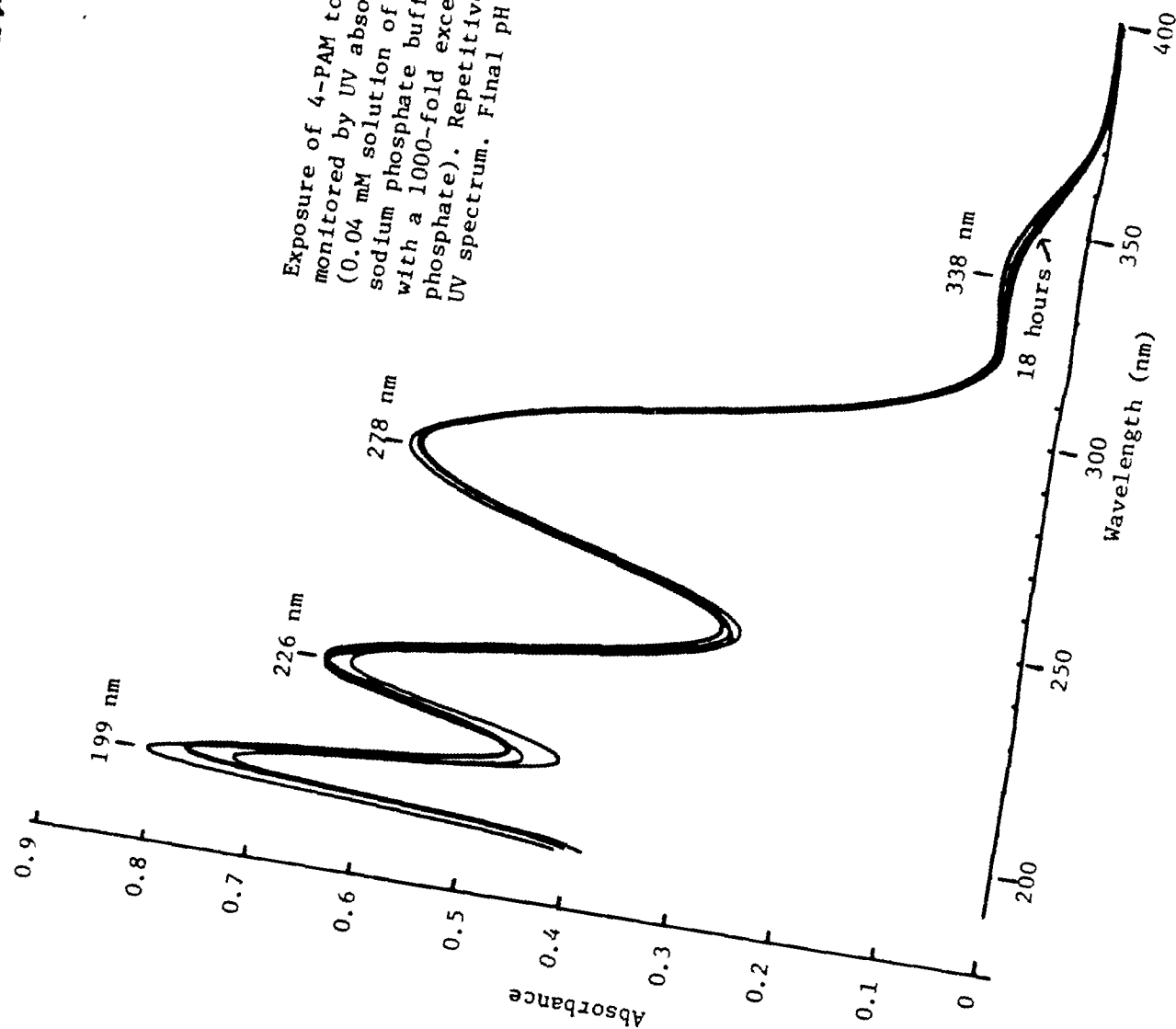
54

Fig. 22

Exposure of 4-PAM to triethyl phosphate monitored by UV absorption spectra. (0.04 mM solution of 4-PAM in 20 mM sodium phosphate buffer (pH 7.40) with a 1000-fold excess of triethyl phosphate). Repetitive scans of the UV spectrum. Final pH 7.40.

Exposure of 3-F-4-PAM to triethyl phosphate monitored by UV absorption spectra. (0.04 mM solution of 3-F-4-PAM in 20 mM sodium phosphate buffer (pH 7.40) with a 1000-fold excess of triethyl phosphate). Repetitive scans of the UV spectrum. Final pH 7.40.
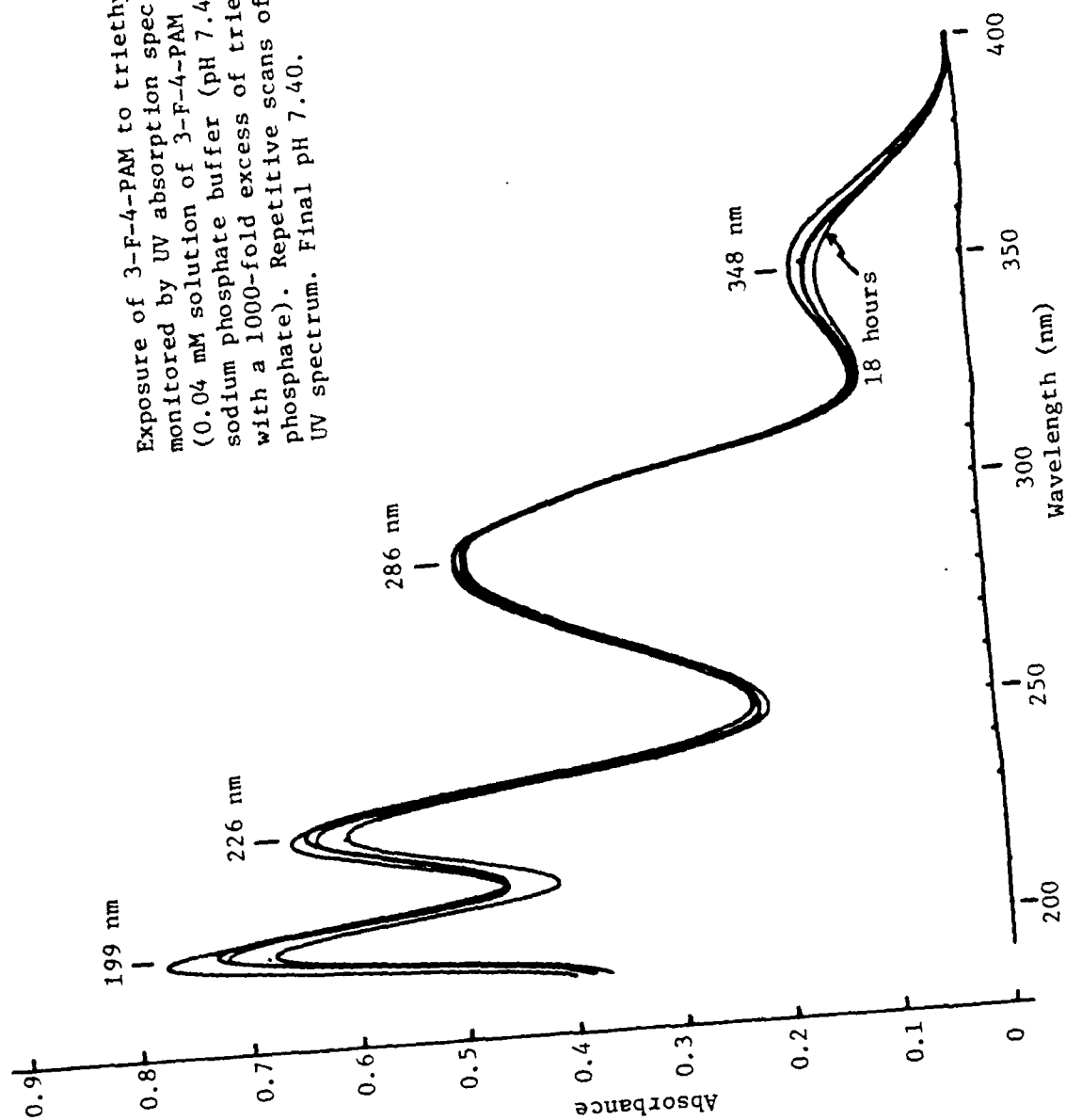
Fig. 23

UV absorption spectra of 4-PAM-benzoate and 3-F-4-PAM-benzoate. (0.02 mM solutions in distilled water, pH 5.9, 1.0 cm path length).
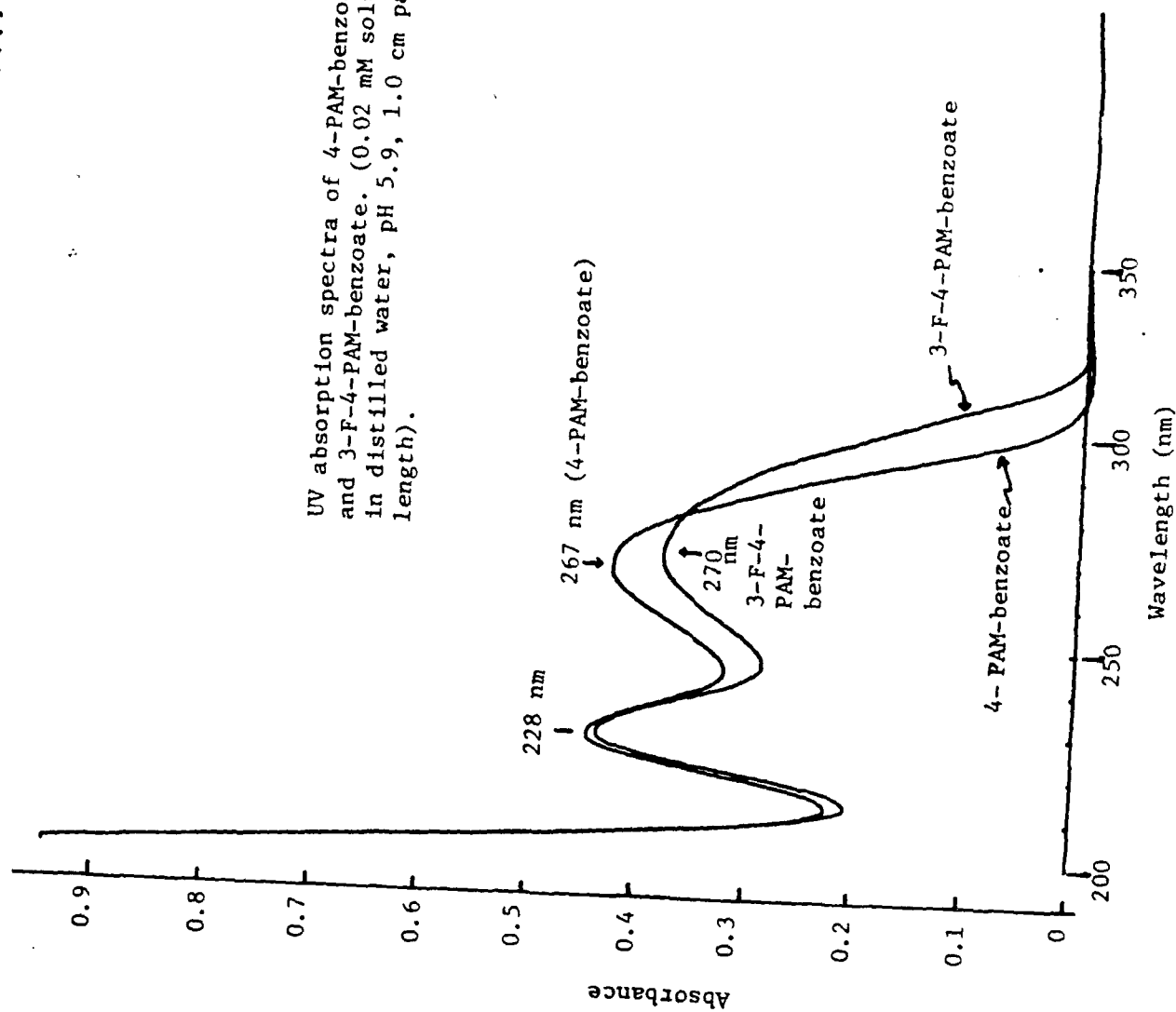
Fig. 24

Fig. 25

Beckman decomposition of 3-F-4-PAM-benzoate monitored by UV absorption spectra. (0.02 mM solution in 10 mM phosphate buffer, pH 8.05). Followed by decay of UV absorption at 270 nm.

Beckman decomposition of 4-PAM-benzoate monitored by UV absorption spectra. (0.02 mM solution in 10 mM phosphate buffer, pH 8.05). Followed by decay of UV absorption at 267 nm.
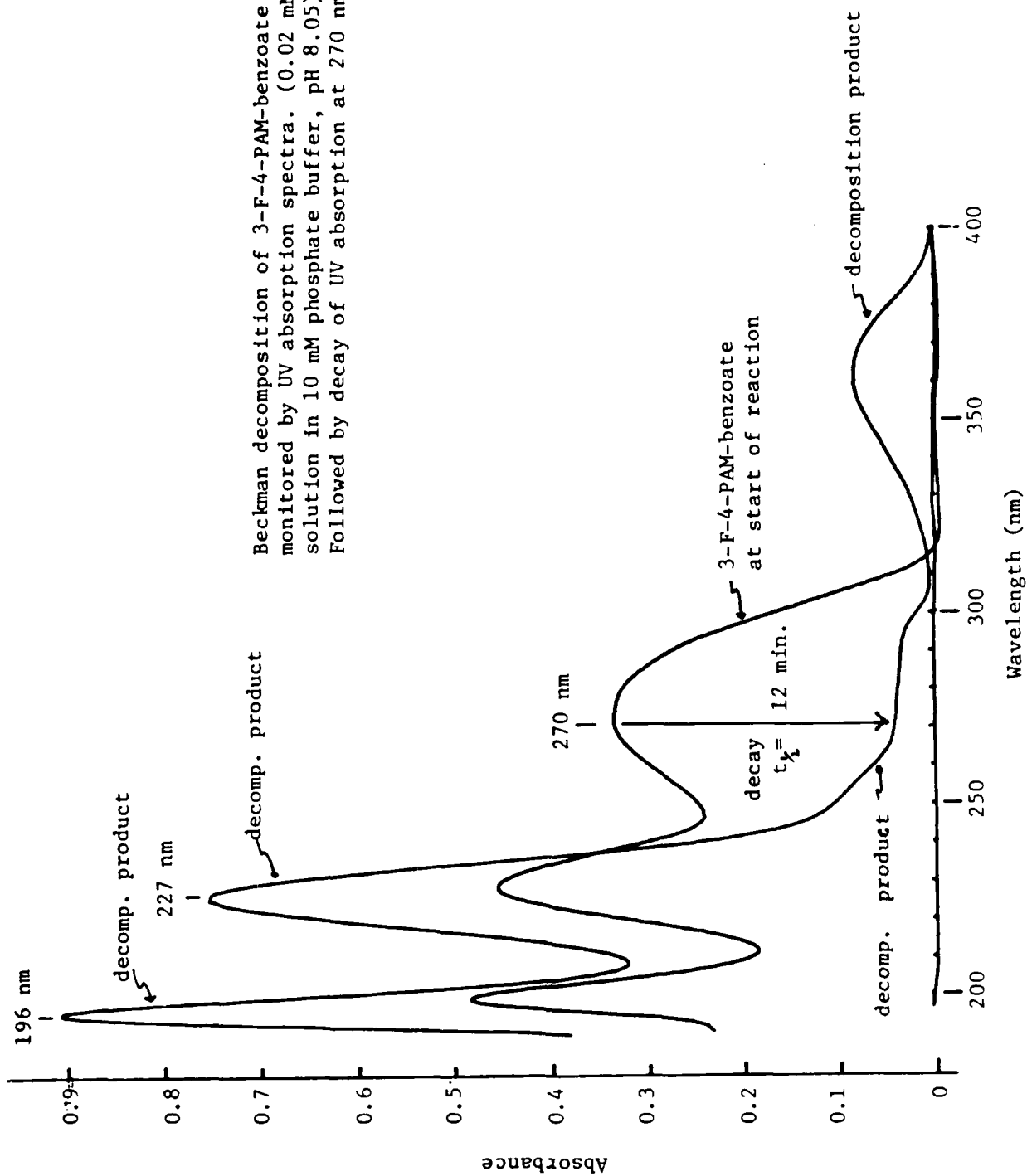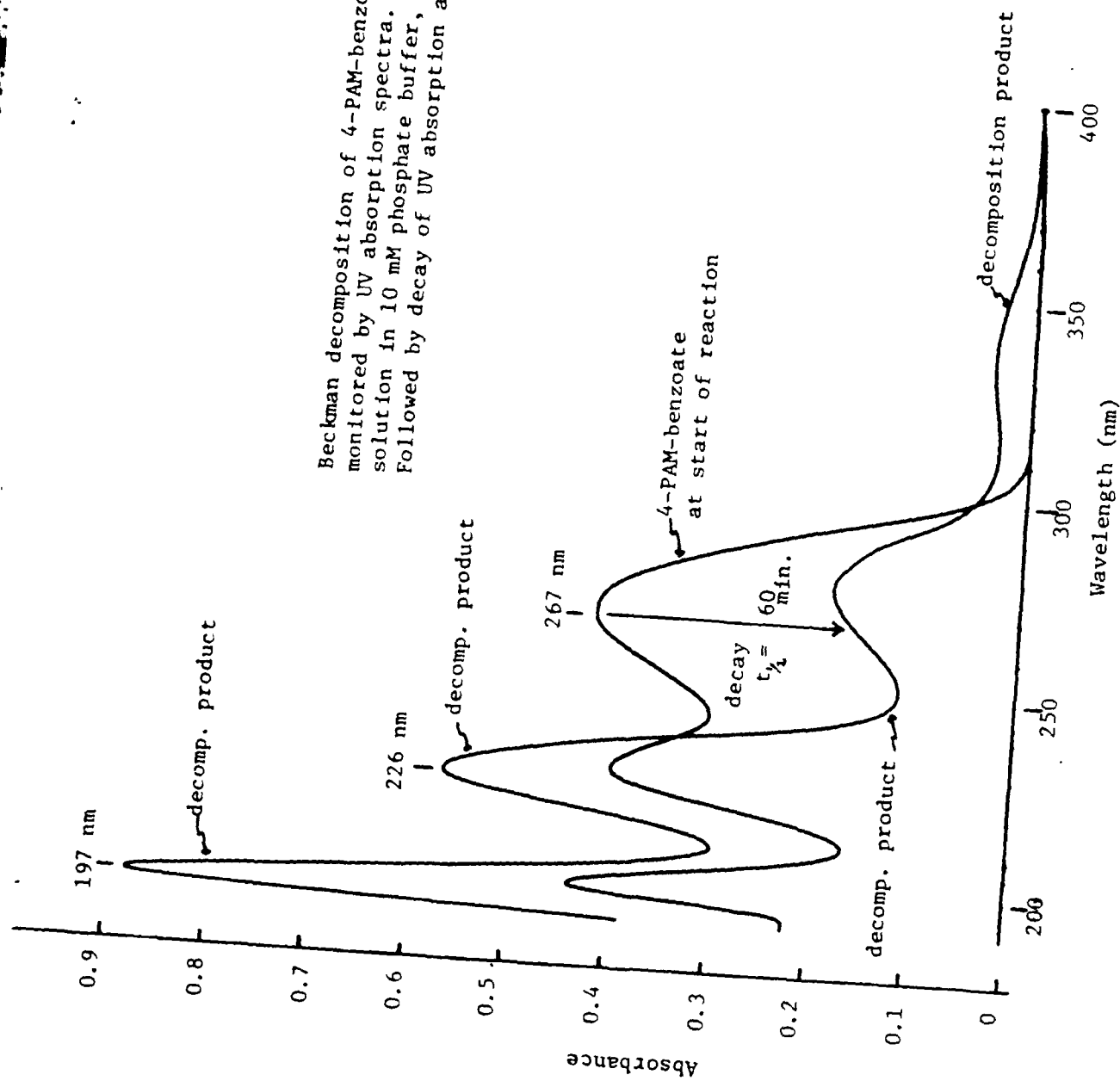
Fig. 26

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